

Bringing Molecules to Life: novel blends of crystallography and electron cryo microscopy (CryoEM) to study virus particle dynamics

Emerging Areas of Biological

Crystallography

Advanced Photon Source

and

Argonne National Laboratory

July 27, 28, 2004

John E. Johnson

Department of Molecular Biology

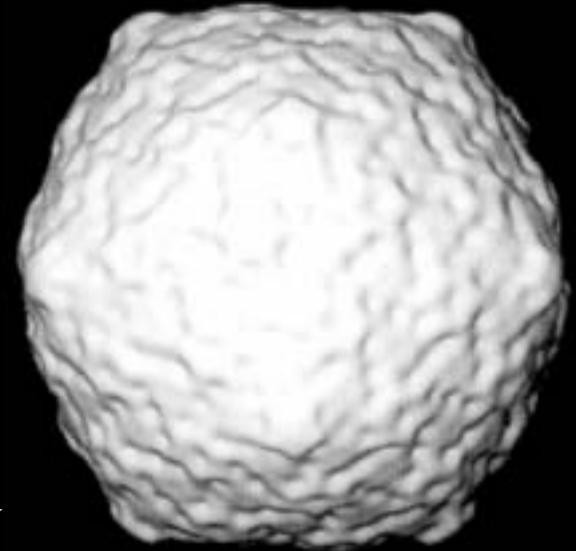
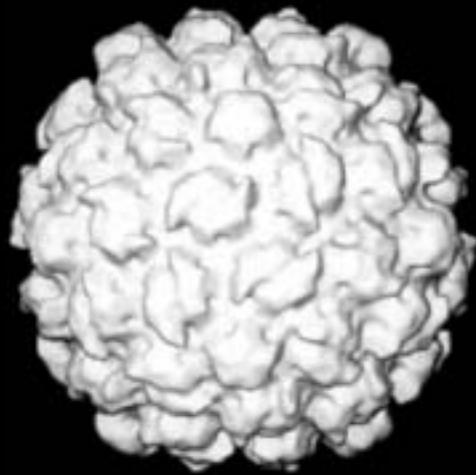
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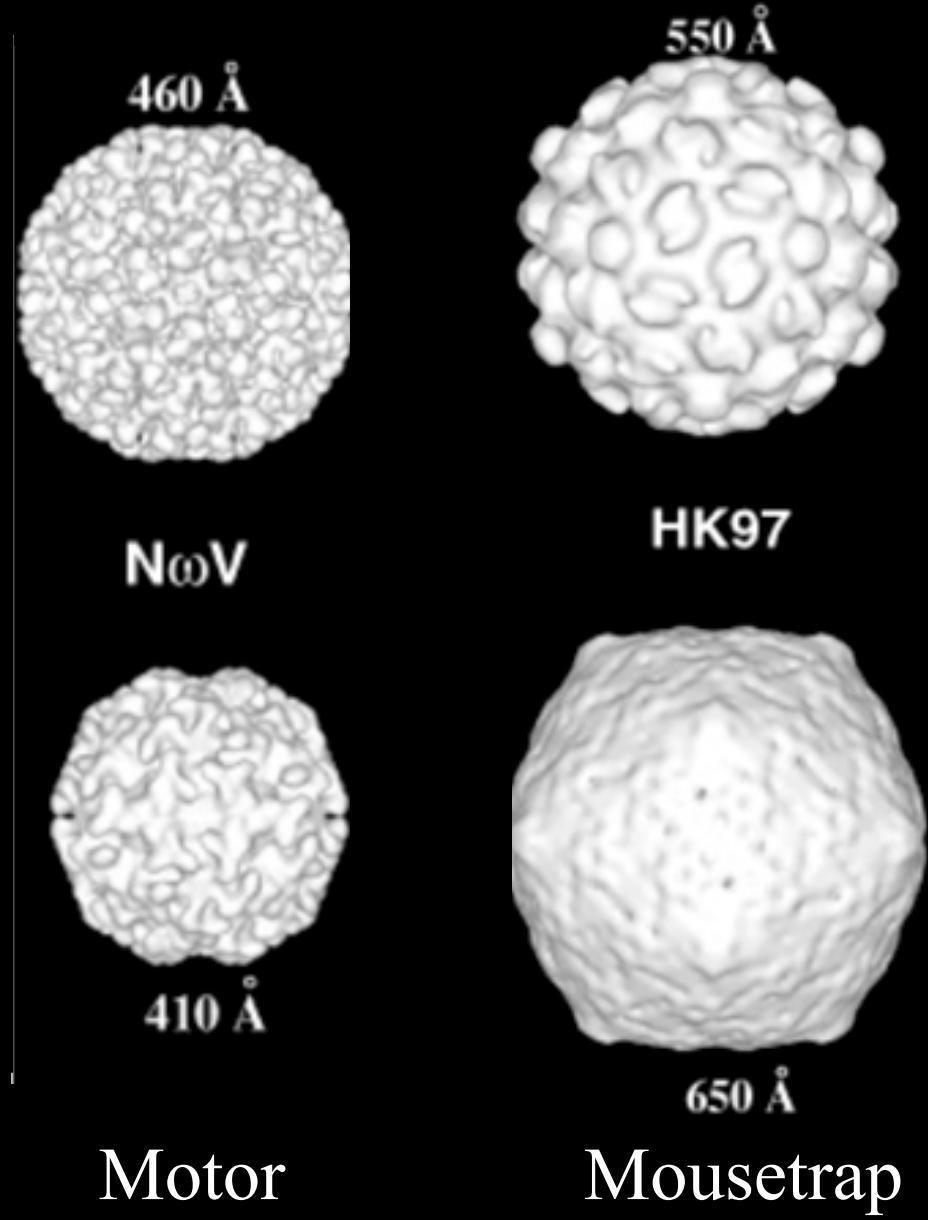
Center for Integrative Molecular Biosciences

The Scripps Research Institute

La Jolla, Ca 92037

jackj@scripps.edu





X-ray Crystal Structure of *Nudaurelia capensis* ω Virus (N ω V)

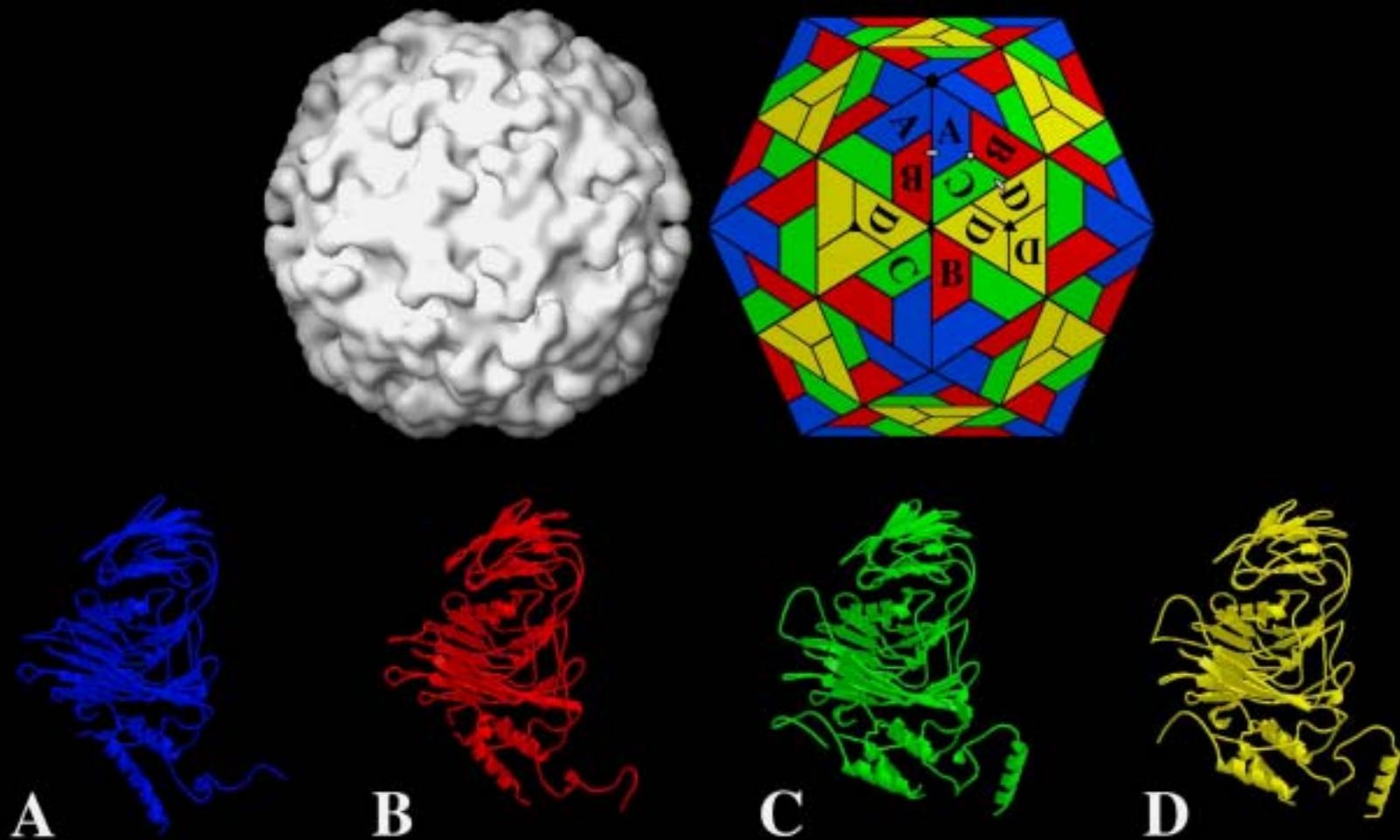
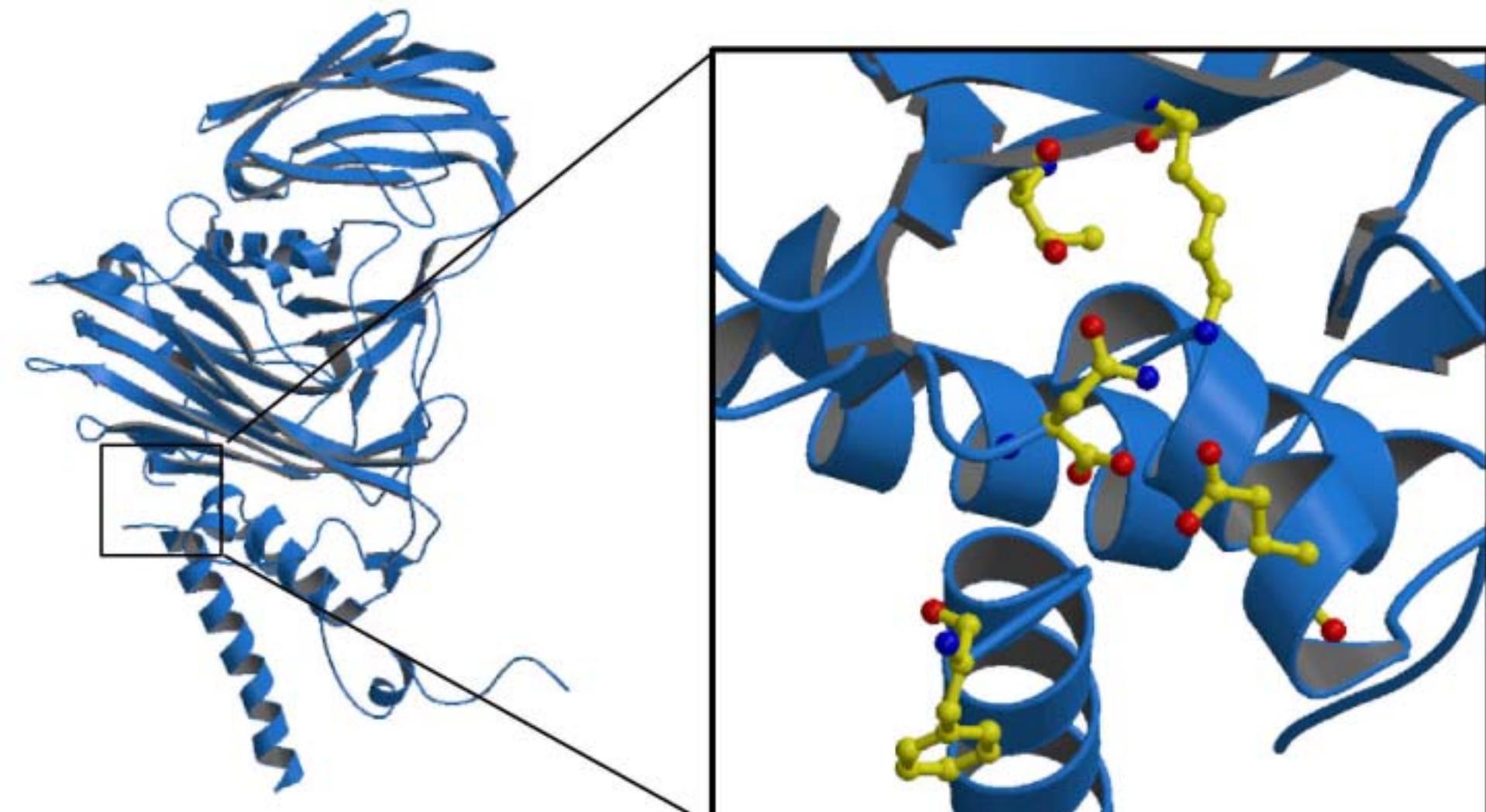


Table 1. Statistics on native data collection and phase refinement for NøV

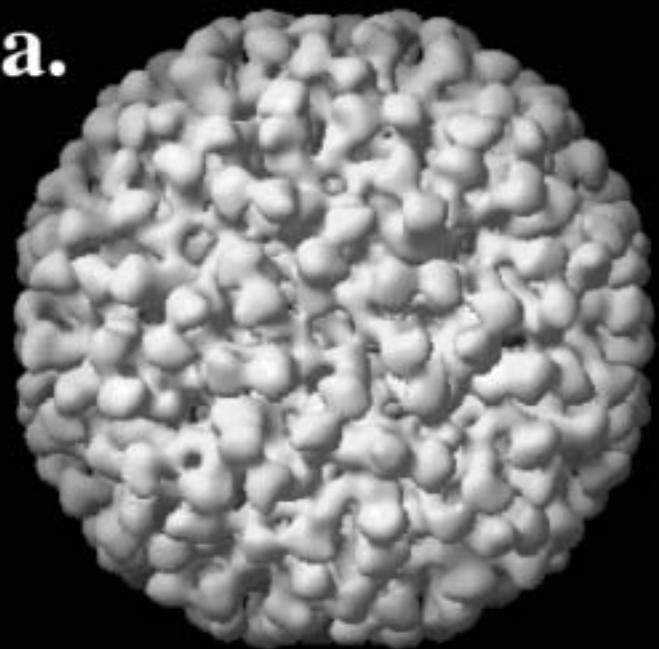
Resolution (Å)	Unique reflections	Observed data (%)	Correlation coefficient
20.0–15.0	73,416	80	0.93
15.0–11.5	78,288	80	0.94
11.5–8.7	206,033	78	0.92
8.7–6.8	255,898	75	0.92
6.8–5.4	555,700	72	0.90
5.4–4.5	468,934	69	0.85
4.5–3.7	623,598	62	0.70
3.7–2.8	203,975	40	0.49
20–2.8	2,465,842	51	0.87
Number of films recorded	500		
Total number of observations	4,141,071		
R_{merge}	12.0%		

Space group P1; Z=1

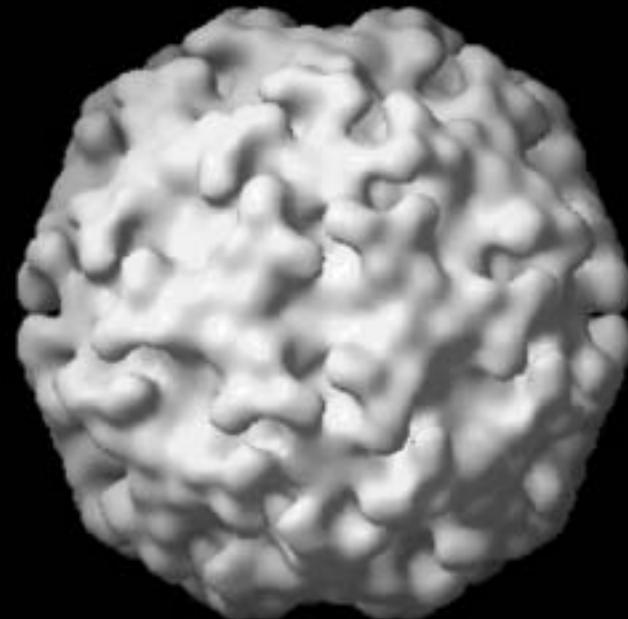
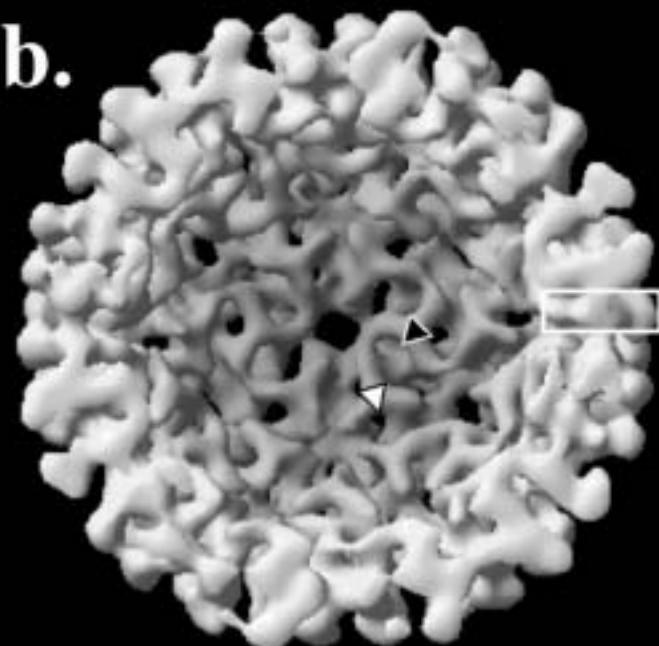
a=414 Å, b=412 Å, c=420 Å, $\alpha=59^\circ$, $\beta=59^\circ$, $\gamma=64^\circ$



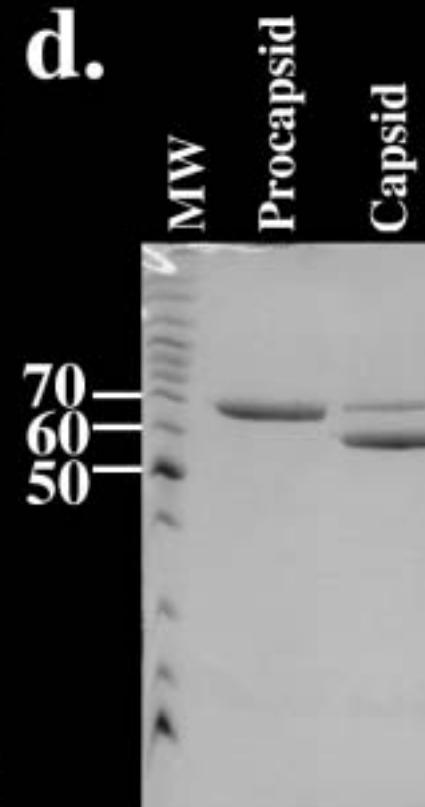
a.



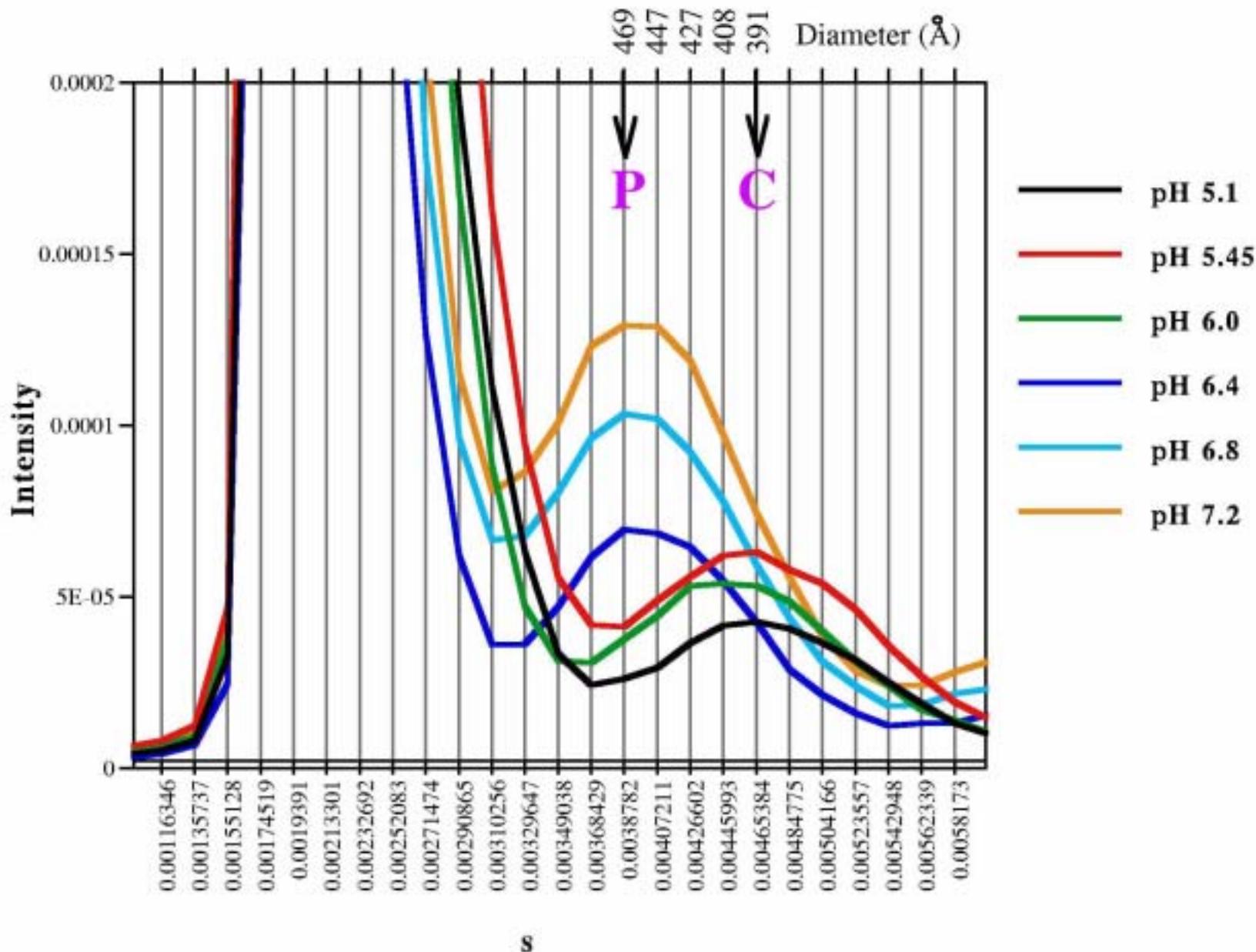
b.



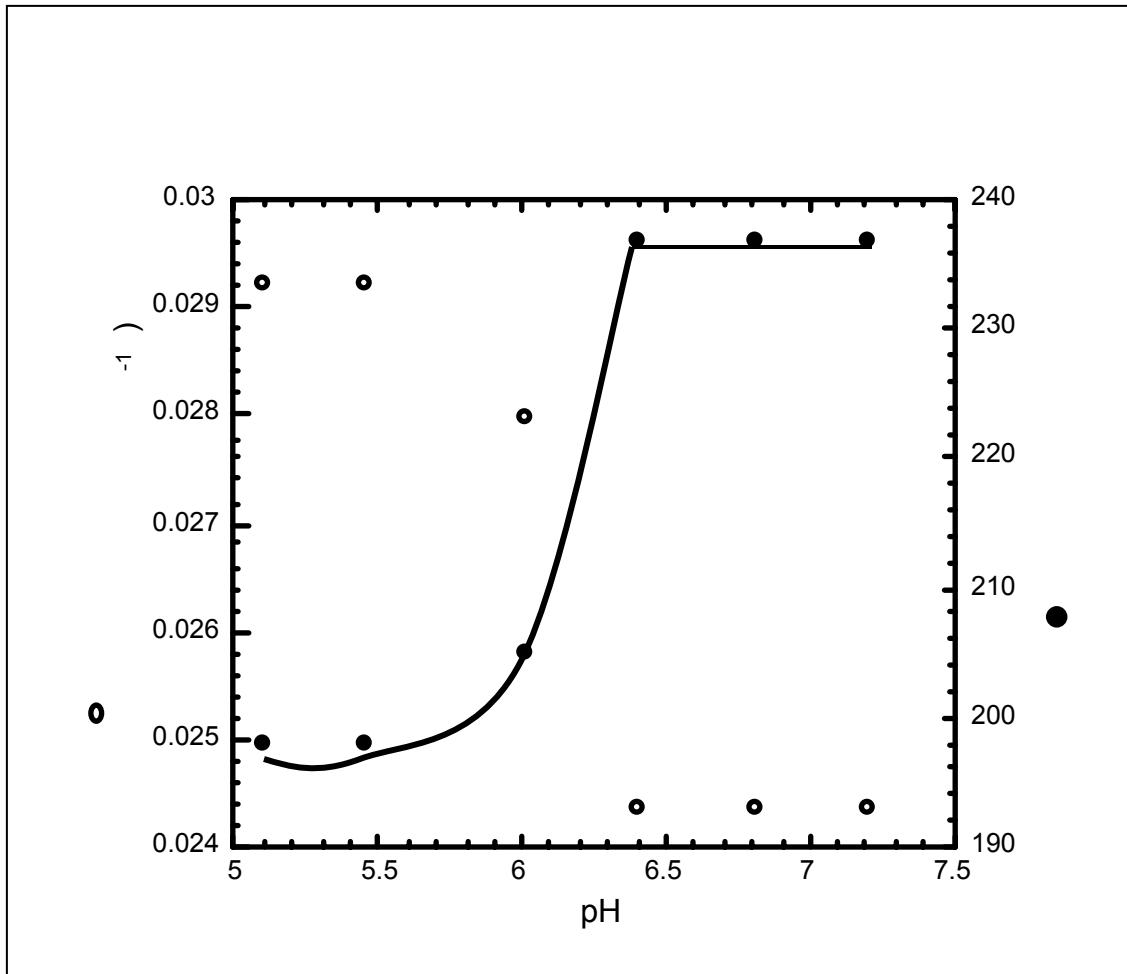
d.



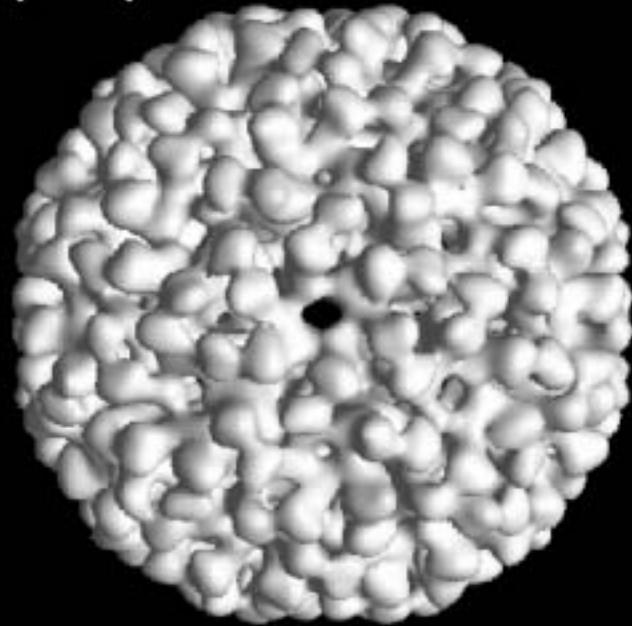
N_oV Step Dialysis Experiment



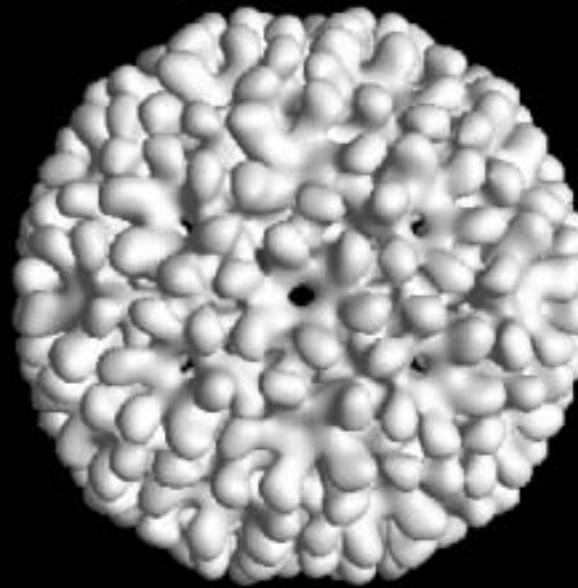
Titration curve of N ω V based on SAX



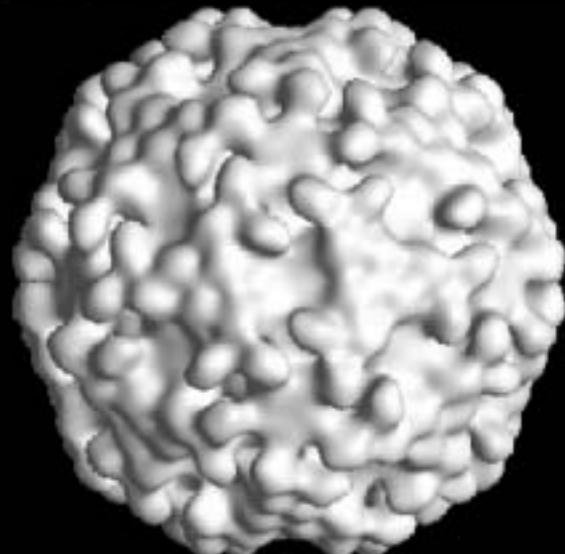
procapsid



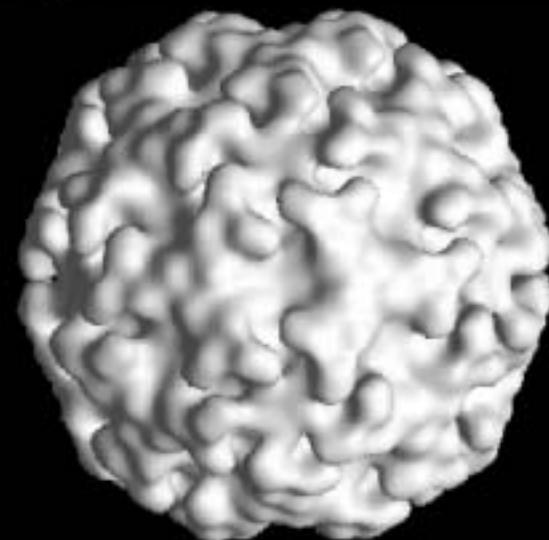
pH5.8 intermediate



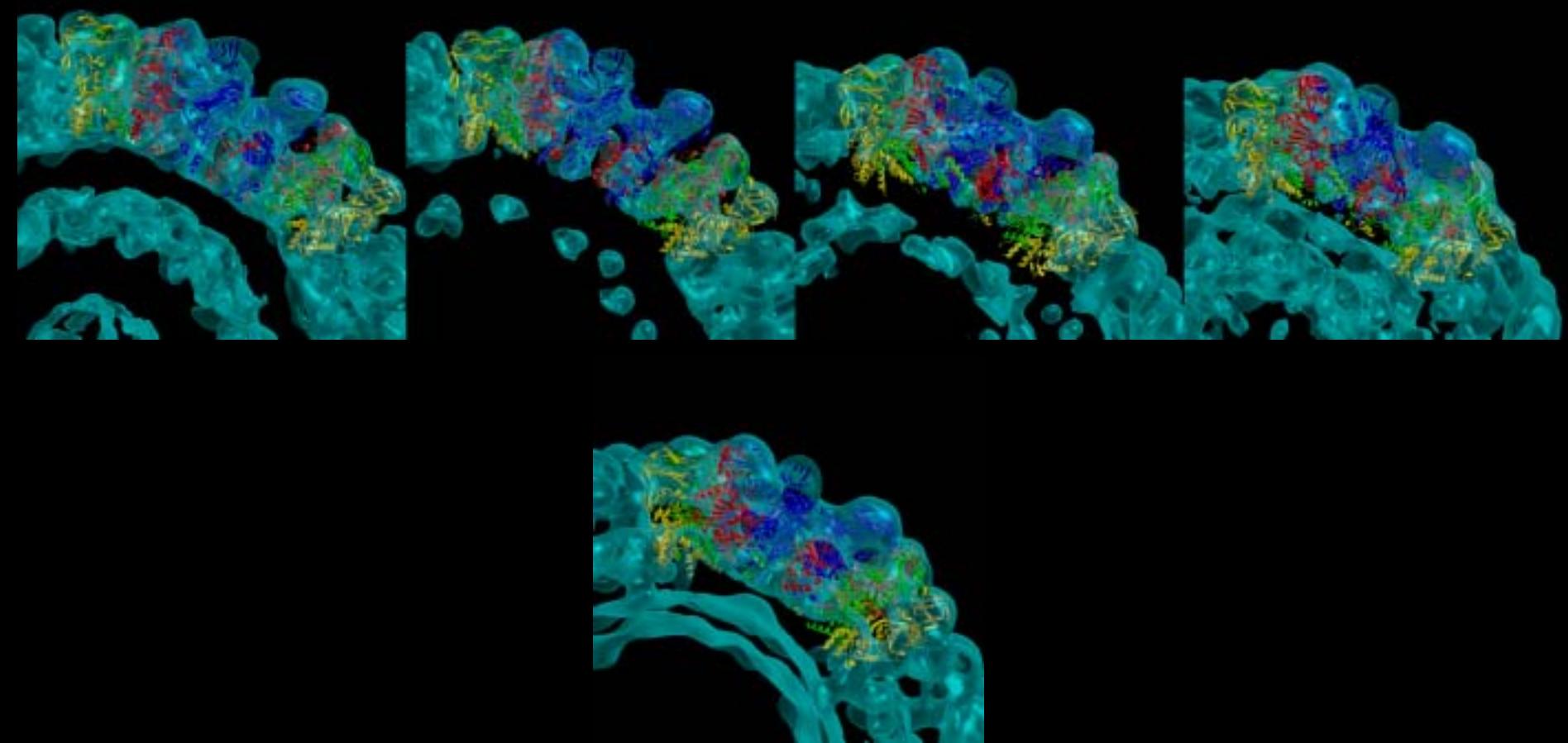
pH5.5 intermediate



capsid

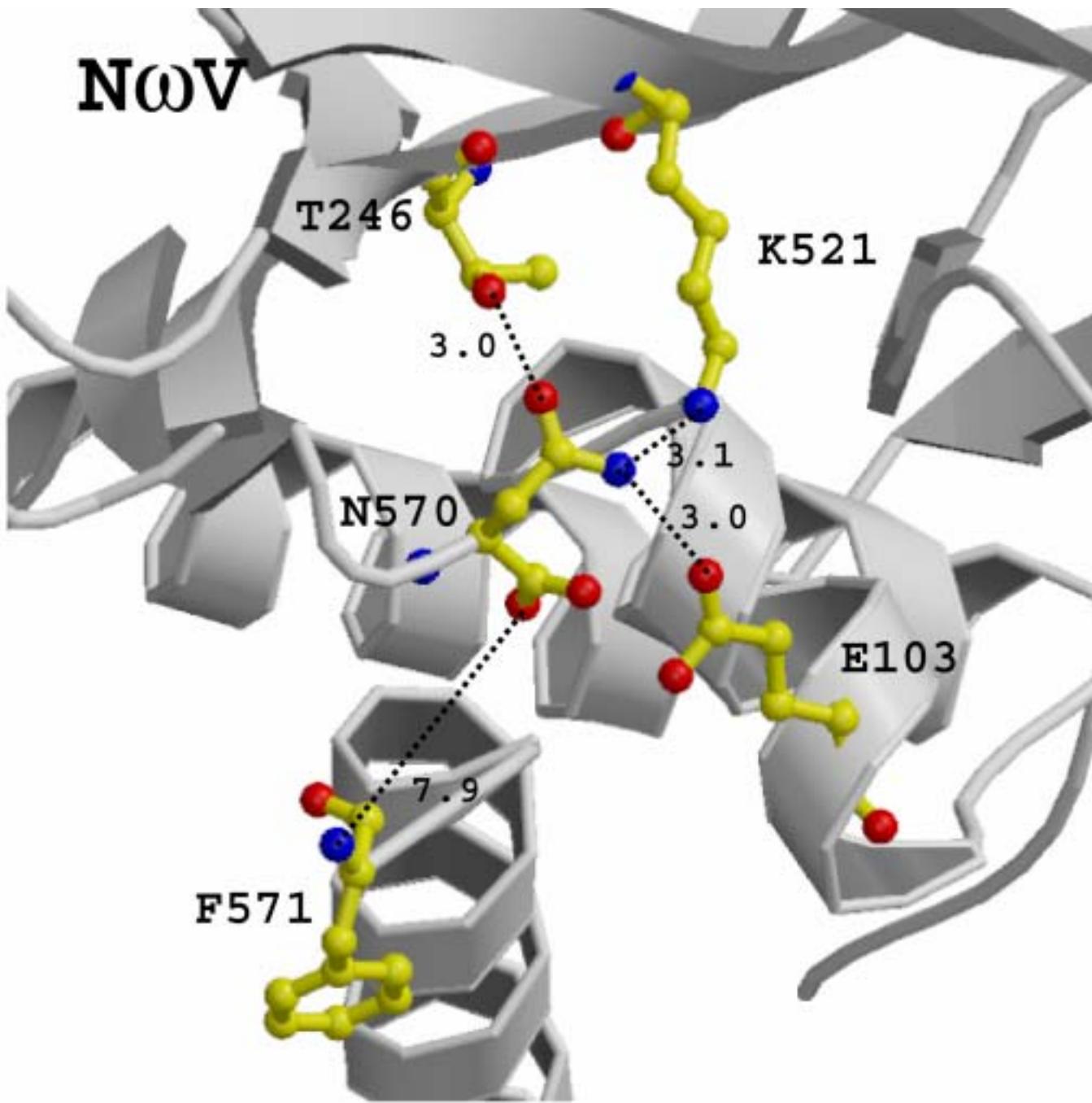


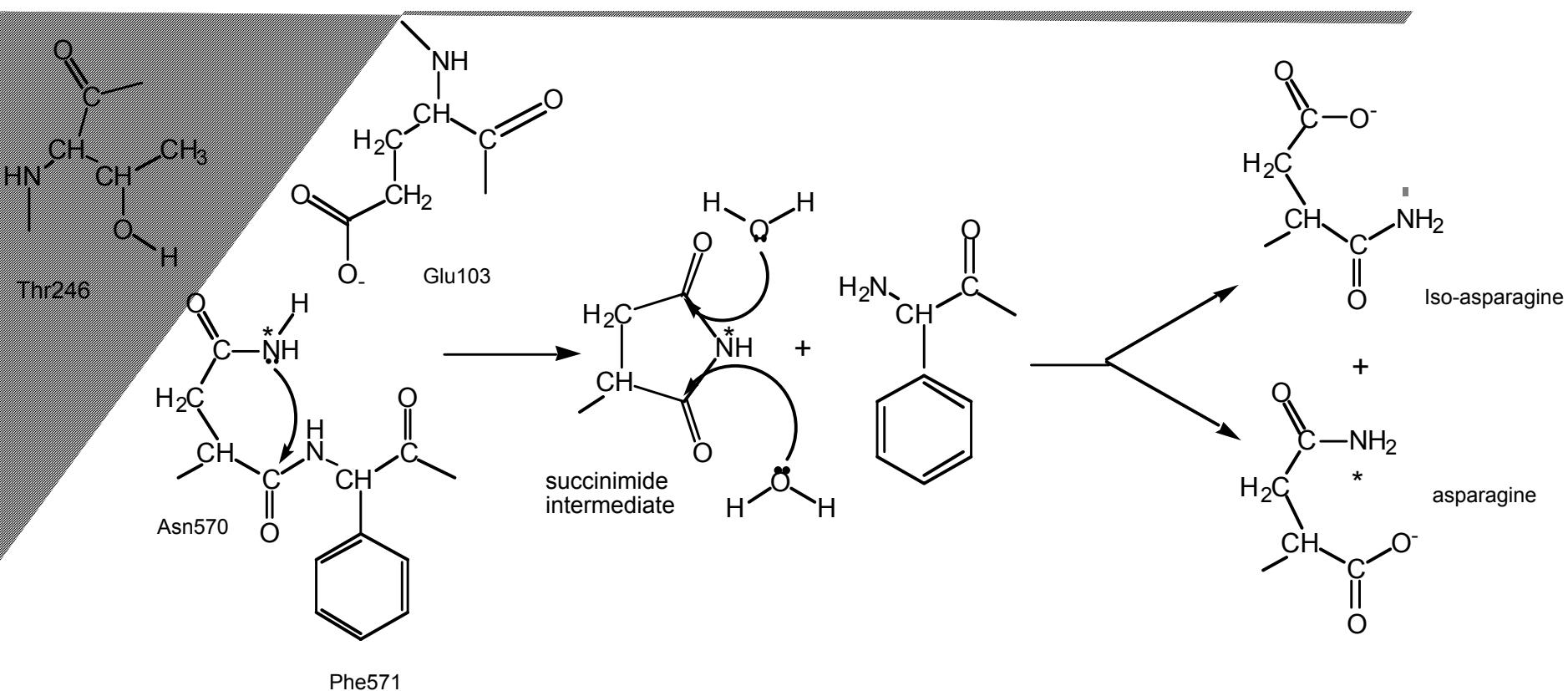
Model fitting

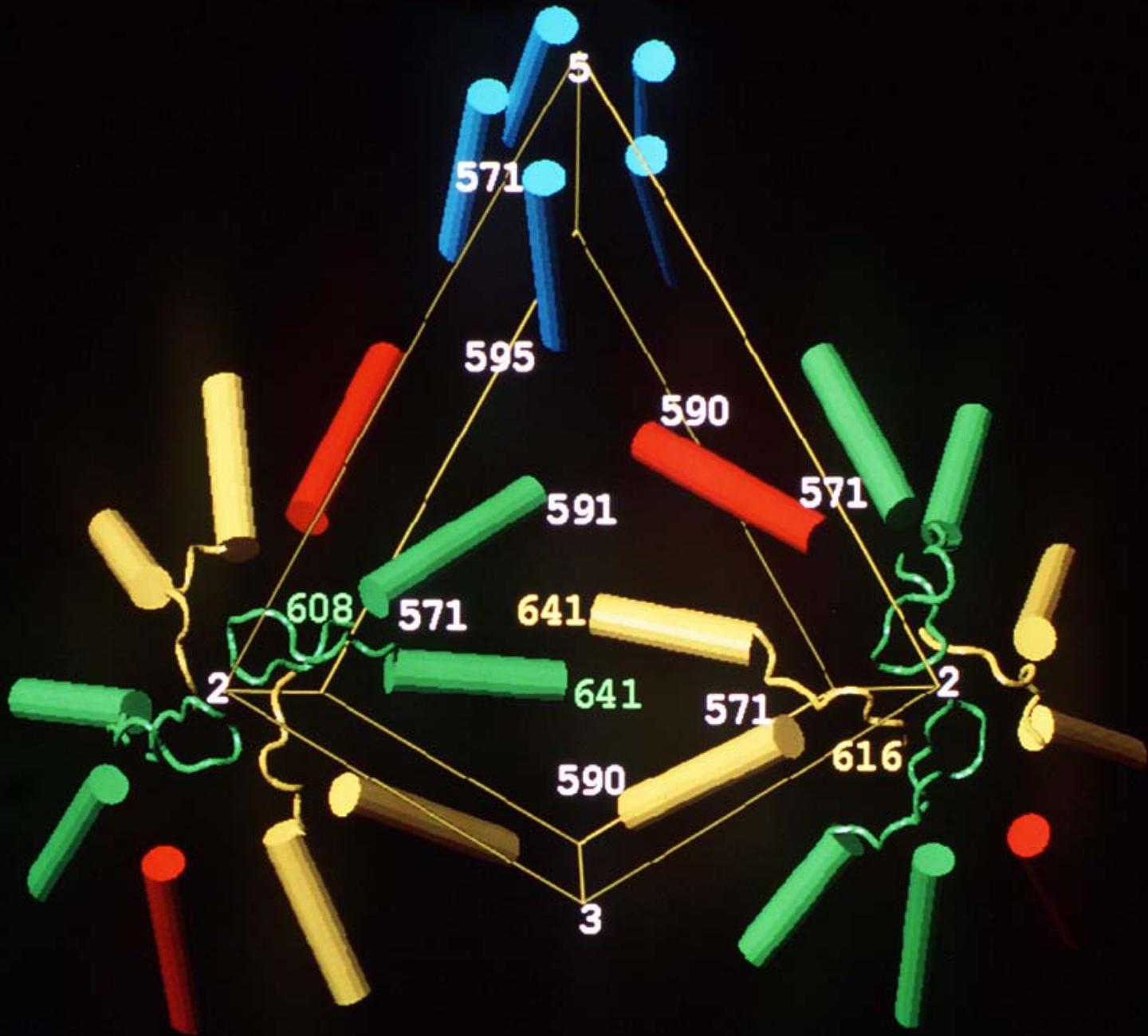


QuickTime™ and a
Video decompressor
are needed to see this picture.

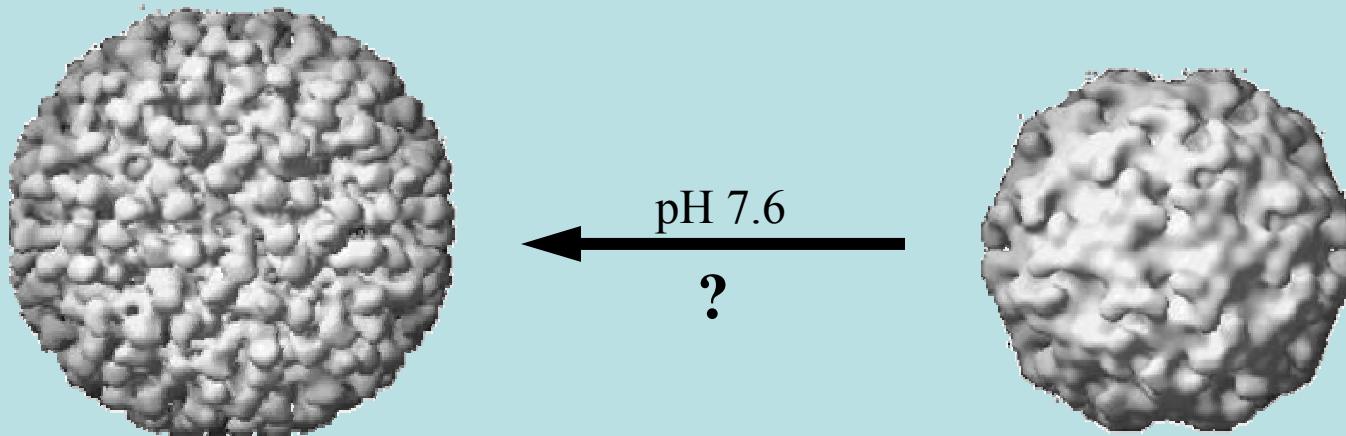
QuickTime™ and a
Video decompressor
are needed to see this picture.



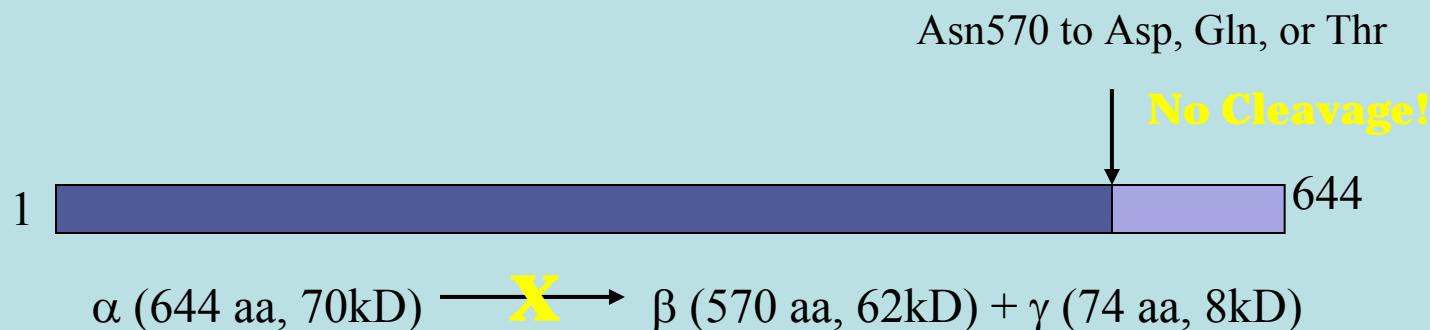




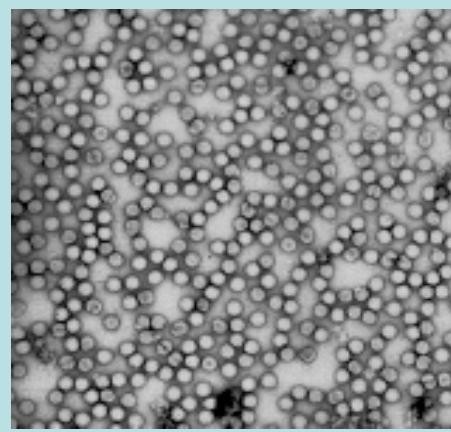
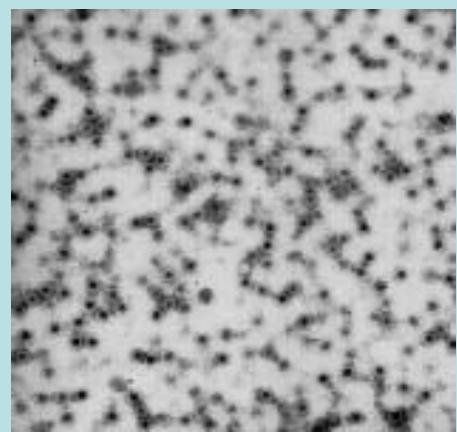
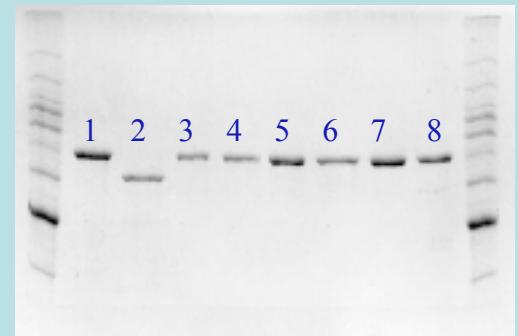
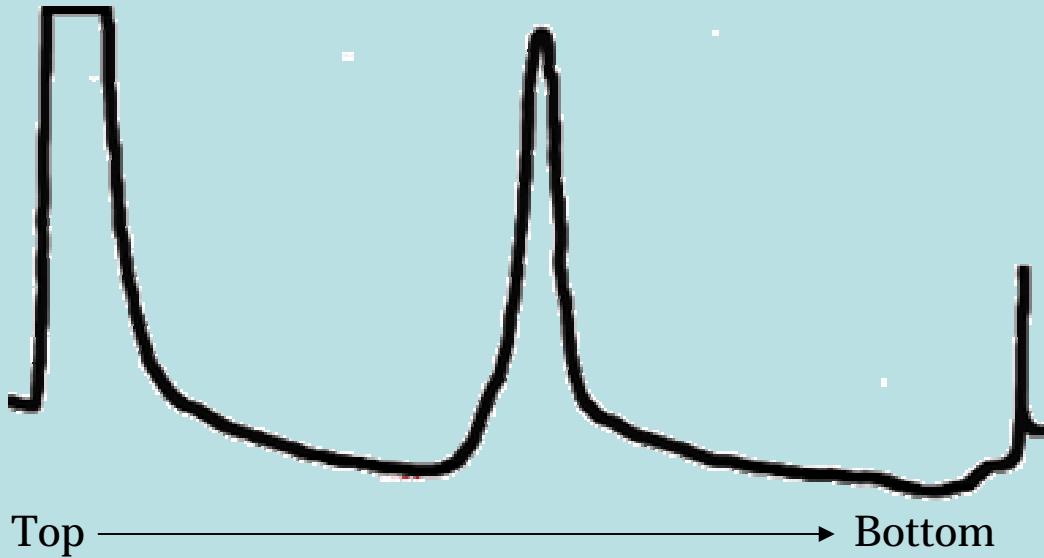
If cleavage is inhibited, is transition reversible?



Cleavage of capsid precursor alpha ($^{1/2}$ life \sim 1 hour):

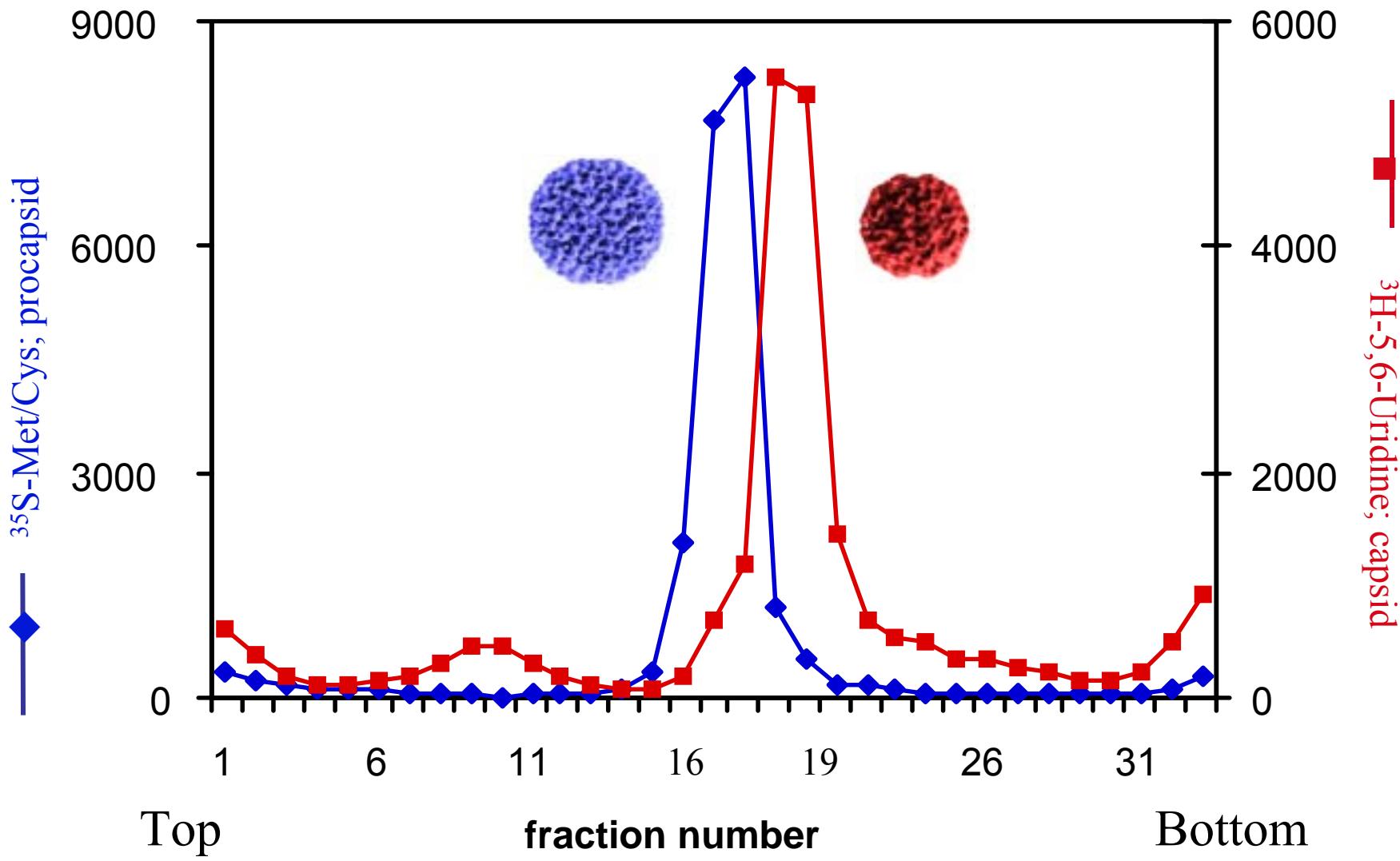


Characterization of N ω V N570T particles.

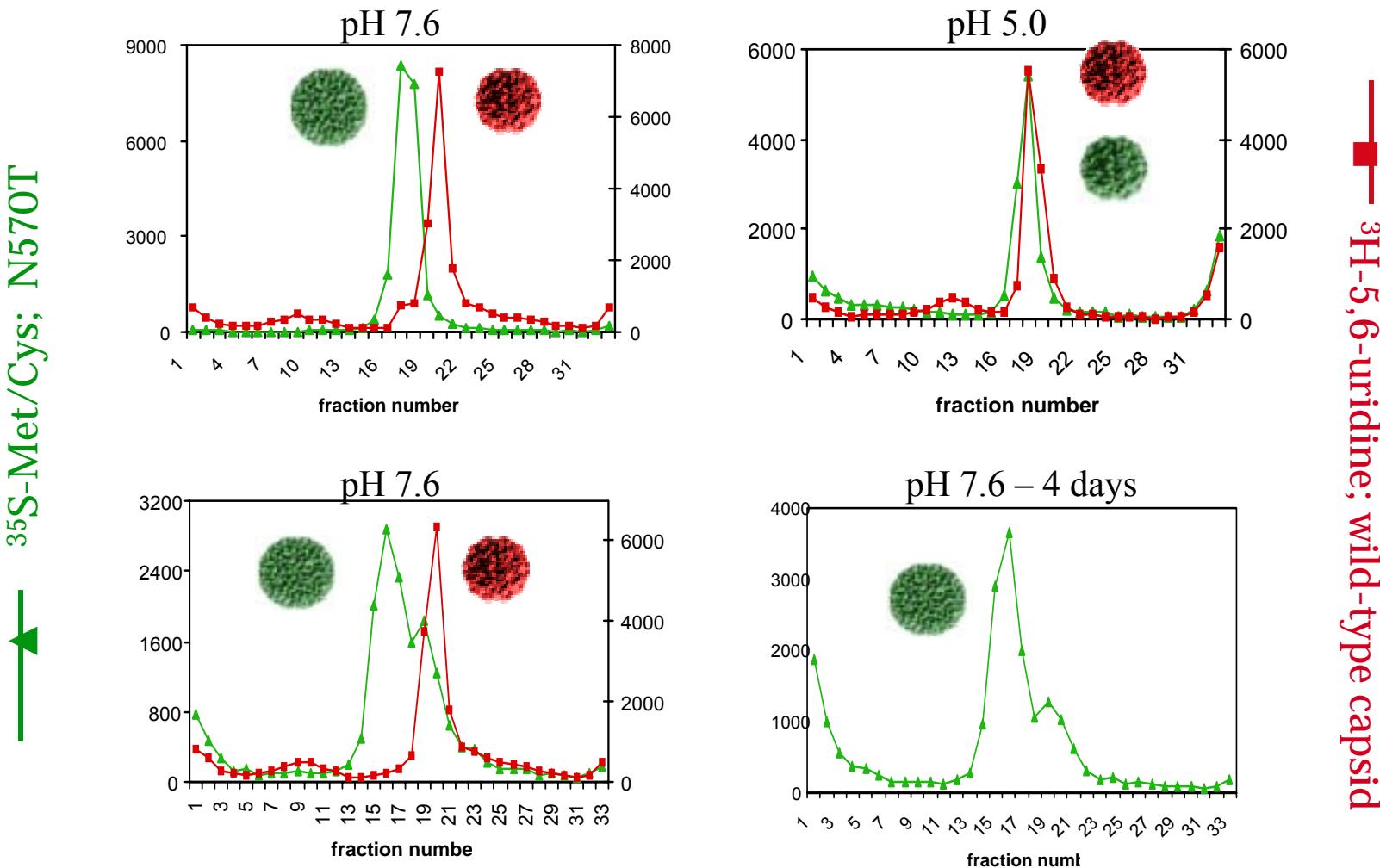


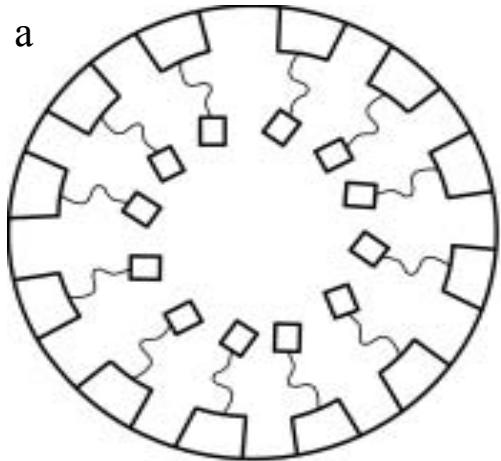
1. wt; pH 7.6
2. wt; pH 5.0
3. N570D; pH 7.6
4. N570D; pH 5.0
5. N570Q; pH 7.6
6. N570Q; pH 5.0
7. N570T; pH 7.6
8. N570T; pH 5.0

N ω V wild-type procapsid + capsid;
10 - 40 % sucrose gradient; pH 7.6

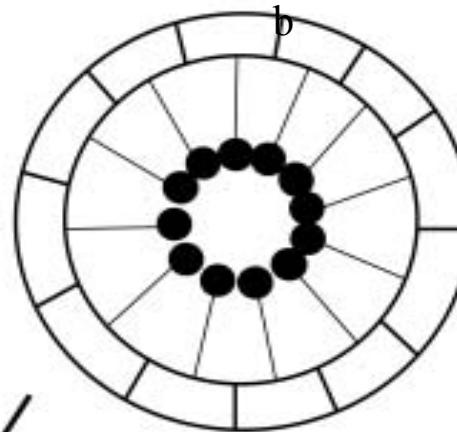


N570T is reversible, but sedimentation rate changes.

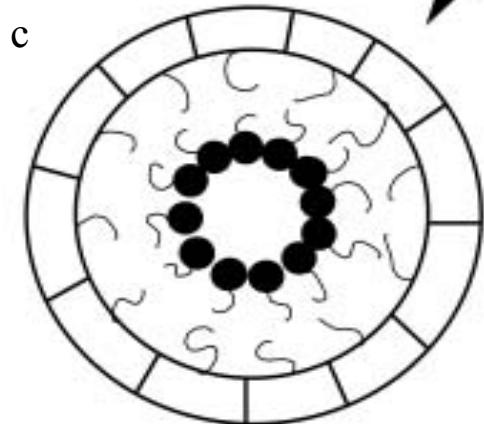




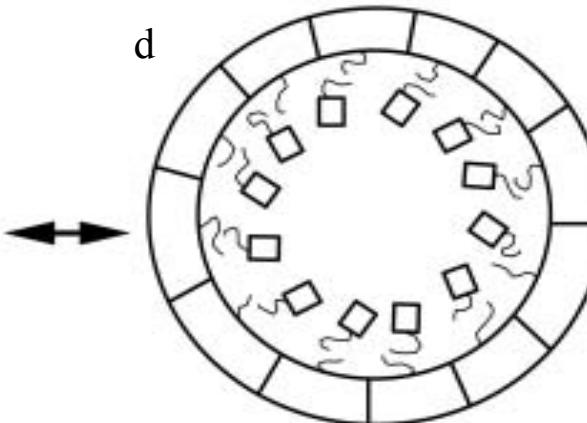
Procapsid;
pH 7.6



Uncleaved
Capsid; pH 5.0



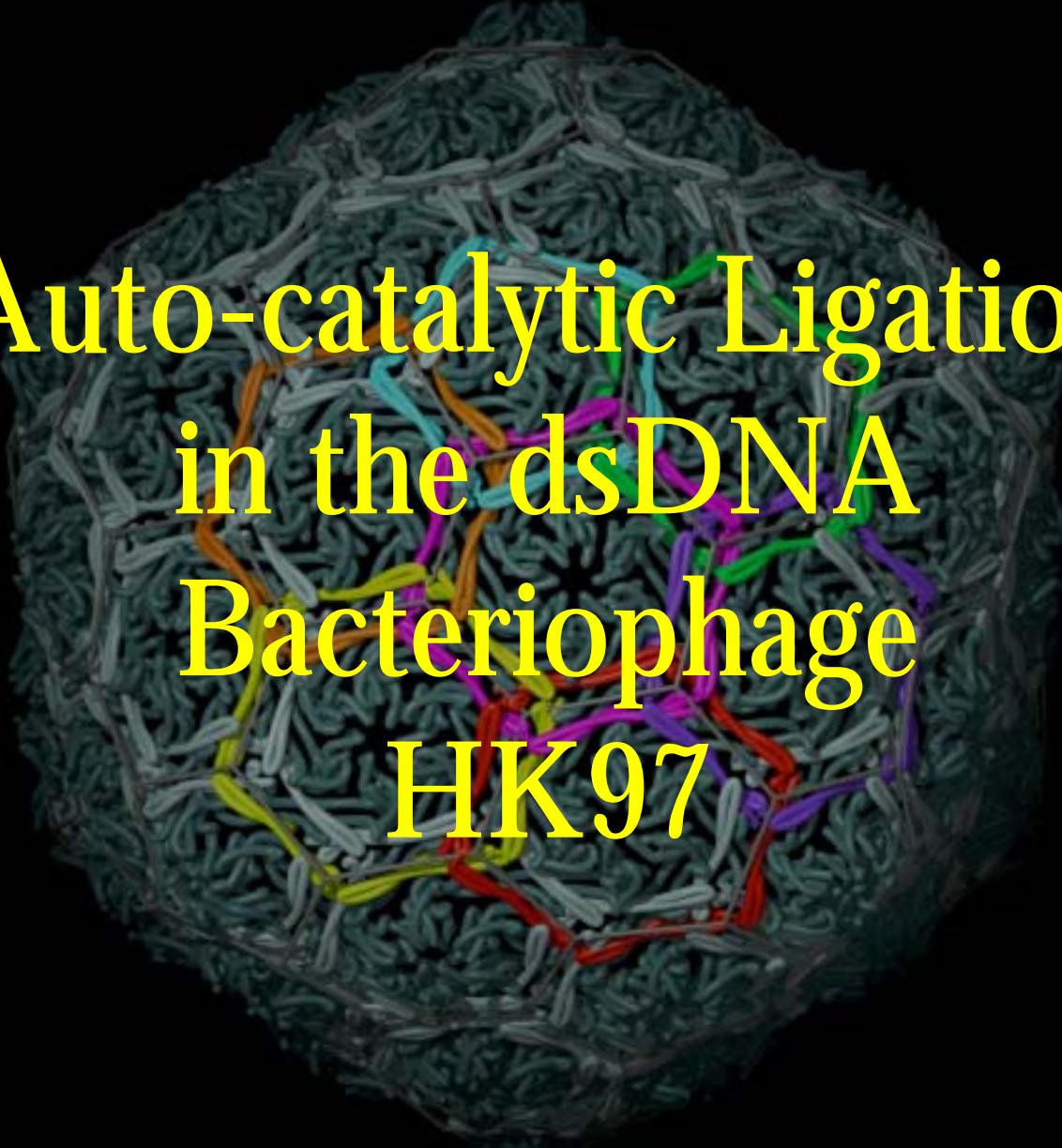
Cleaved Capsid;
pH 5.0



Cleaved Capsid;
pH 7.6

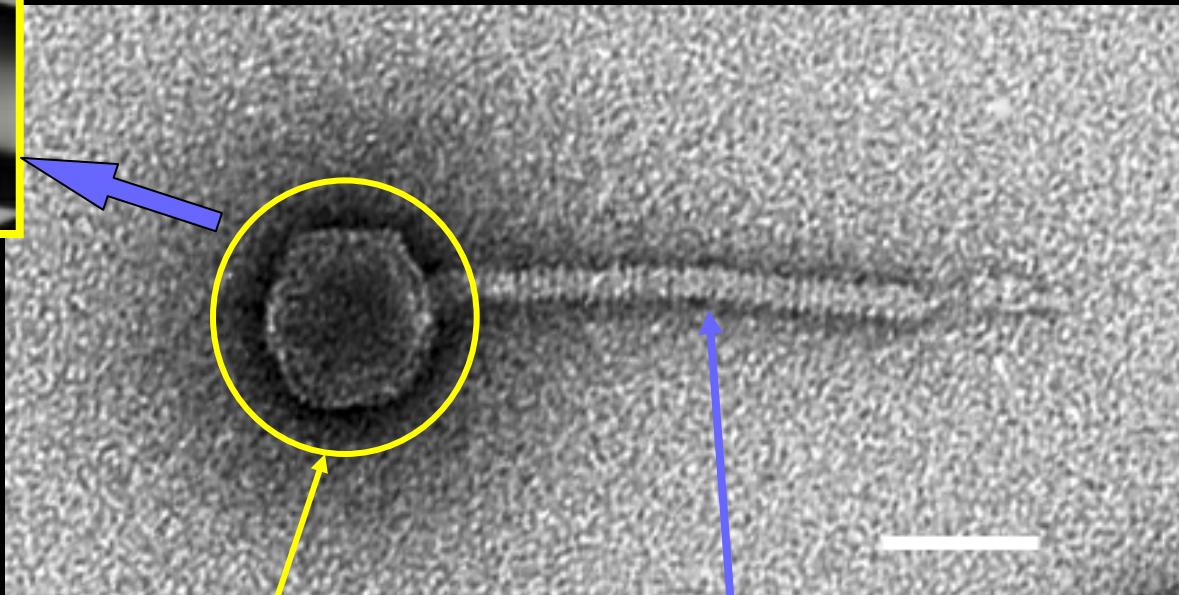
N ω V References

- Agrawal D, Johnson J. 1995. Assembly of the T=4 Nudaurelia capensis omega virus capsid protein, post translational cleavage, and specific encapsidation of its mRNA in a baculovirus expression system. *Virology* 207:89-97
- Munshi S, Liljas L, Cavarelli W, Bomu W, McKinney B, Reddy V, Johnson J. 1996. The 2.8Å structure of *T*=4 animal virus and its implications for membrane translocation of RNA. *J. Mol. Biol.* 261:1-10
- Canady MA, Tihova M, Hanzlik TN, Johnson JE, Yeager M. 2000. Large Conformational Changes in the Maturation of a Simple RNA Virus, Nudaurelia capensis omega Virus (N ω V). *J Mol Biol* 299:573-584
- Canady M, Tsuruta H, Johnson J. 2001. Analysis of Rapid, Large-Scale Protein Quaternary Structural Changes: Time-Resolved X-ray Solution Scattering of Nudaurelia capensis ω Virus (N ω V) Maturation. *J. Mol. Biol.* 311:803-814
- Taylor, D. J., Krishna, N. K., Canady, M. A., Schneemann, A., and Johnson, J. E. 2002. Large-scale, pH-dependent, quaternary structure changes in an RNA virus capsid are reversible in the absence of subunit autoproteolysis. *J Virol* 76:9972-80.
- Lee, K. K., Tang, J., Taylor, D., Bothner, B., and Johnson, J. E. 2004. Small compounds targeted to subunit interfaces arrest maturation in a nonenveloped, icosahedral animal virus. *J Virol* 78:7208-16.



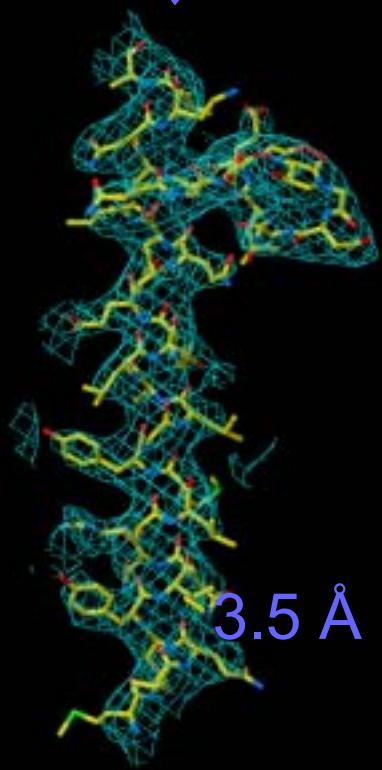
Auto-catalytic Ligation in the dsDNA Bacteriophage HK97

HK97 Negative Stain EM



Head

Tail



3.5 Å x-ray map

HK97 Head II Structure Determination

Space Group P2₁

Lattice Constants a=581Å, b=628Å, c= 789Å, β=89.9°

Data Set Recorded on Bio Cars beam line 14BM-C APS

□□□□□ of Images Recorded (Mar 345) 760 (osc ang 0.25°)

Number of Reflections Measured 21,863,976

Number of Unique Reflections 4,800,765

Completeness of Data 90Å-3.4Å 63%

Minimum Partial Accepted 0.5

Rmerge 16%

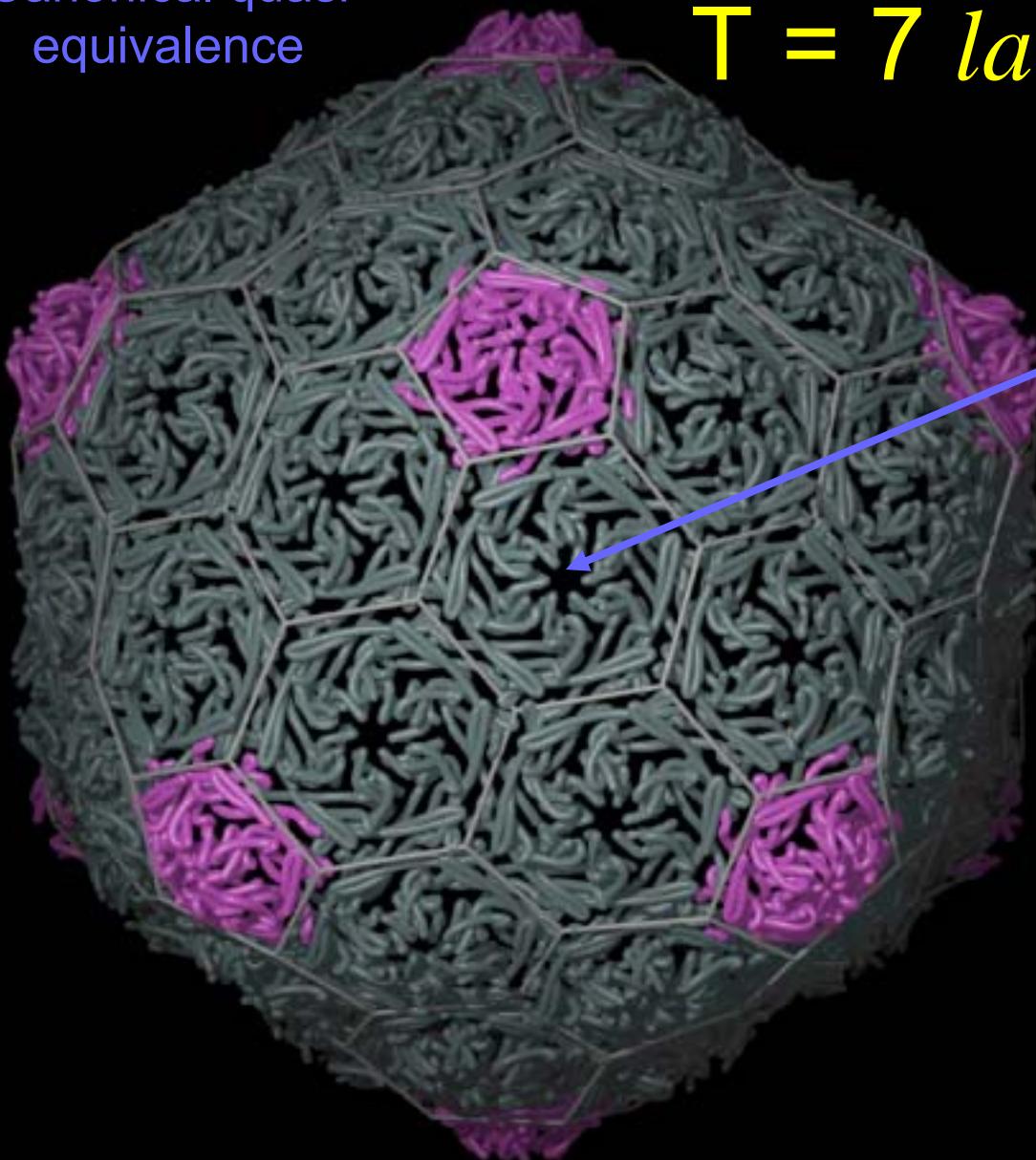
MR Rfactor 32% (Real Space averaging and phase extension
from 20Å to 3.4Å; 60-fold redundancy)

MR CC 0.85

Quasi Symmetry

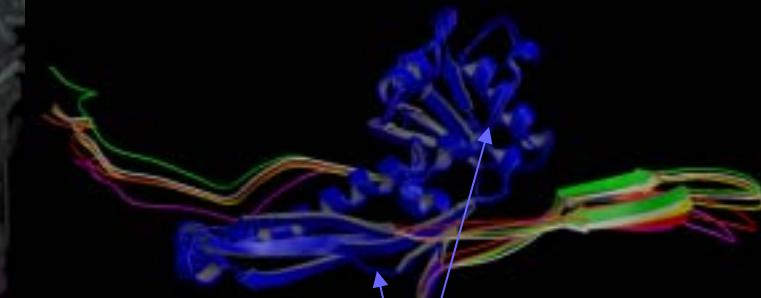
$T = 7$ laevo

Canonical quasi equivalence



High fidelity hexamer symmetry

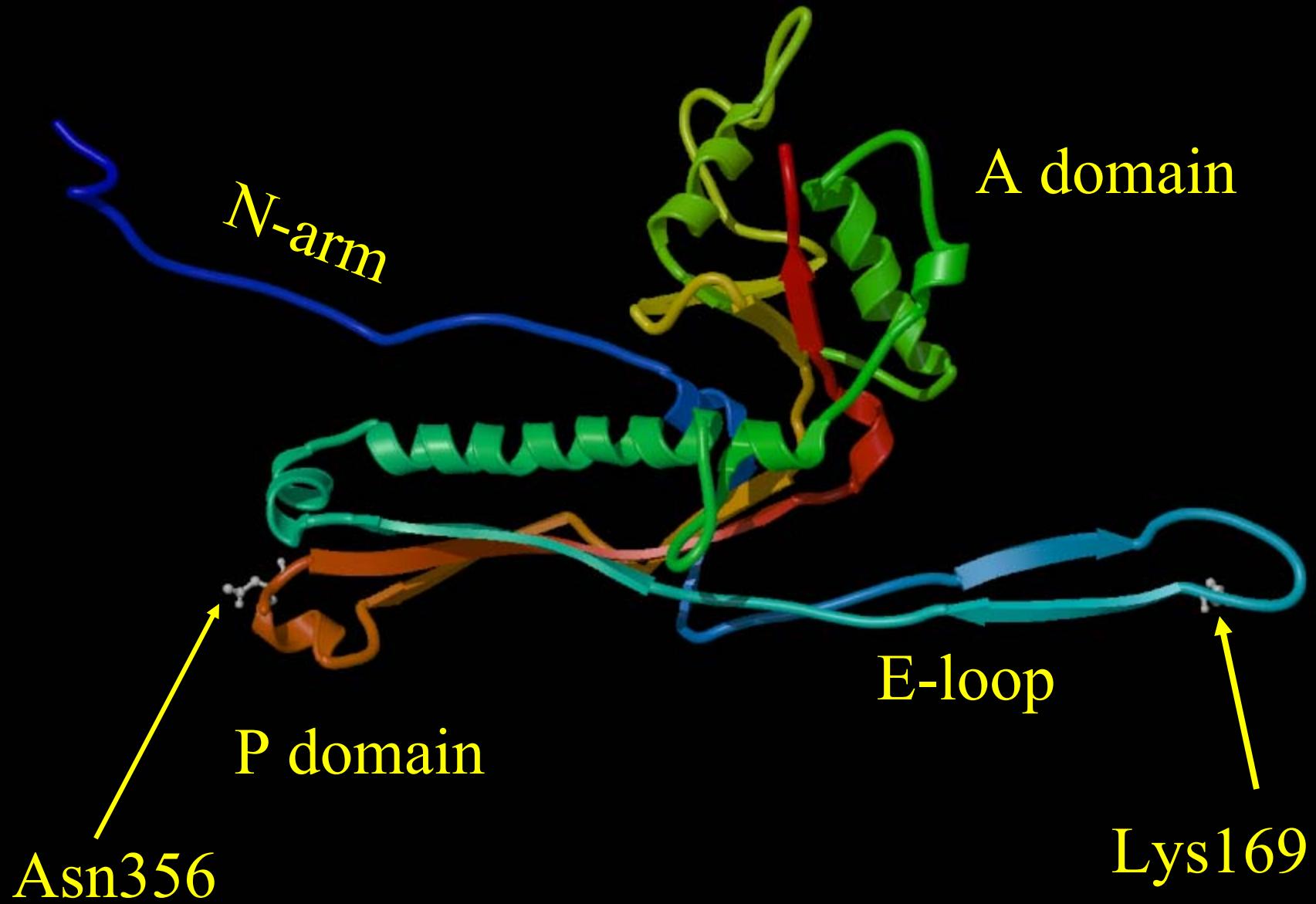
7 superimposed Subunits

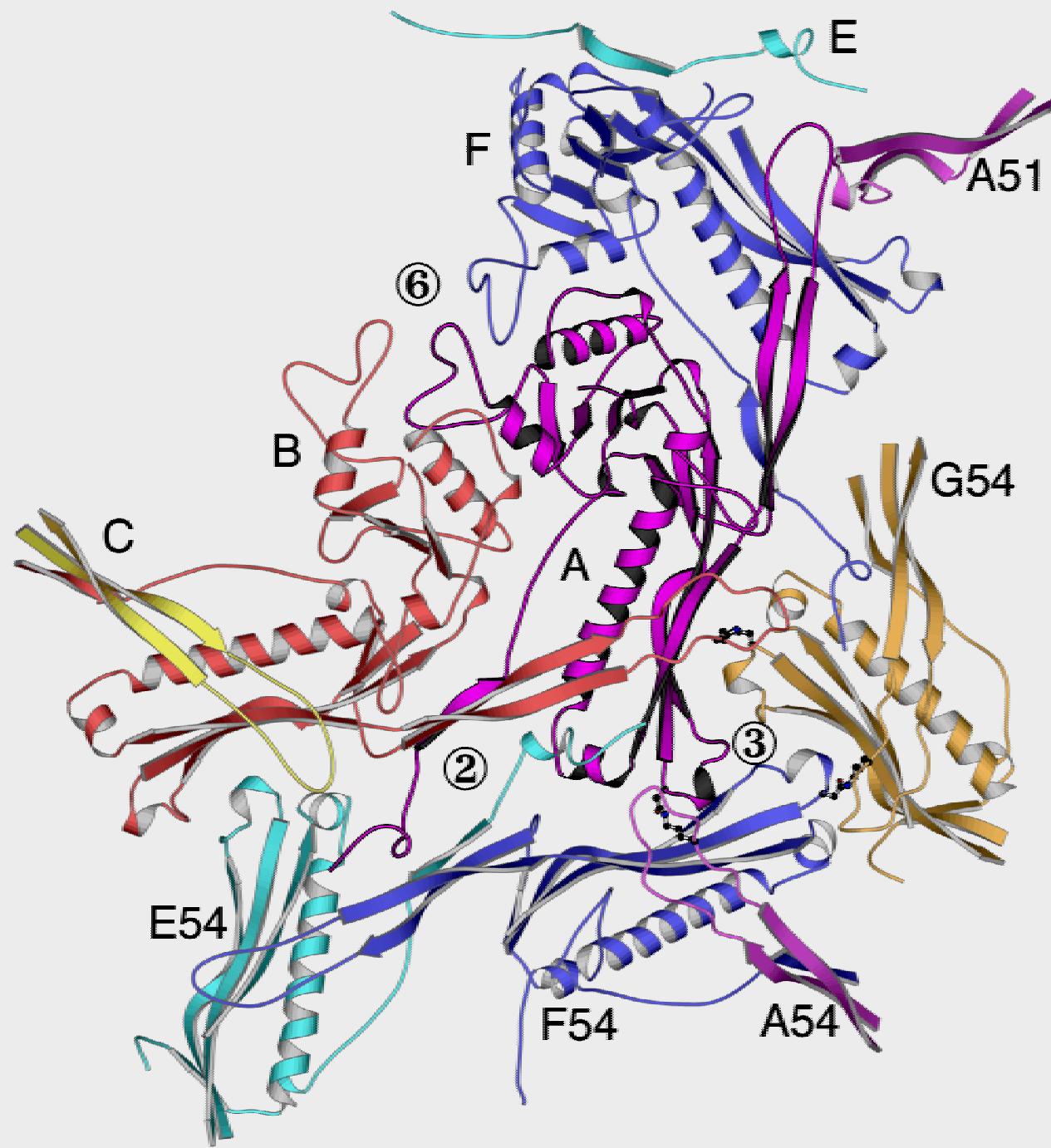


Core Domains (blue) of Subunit Superimpose

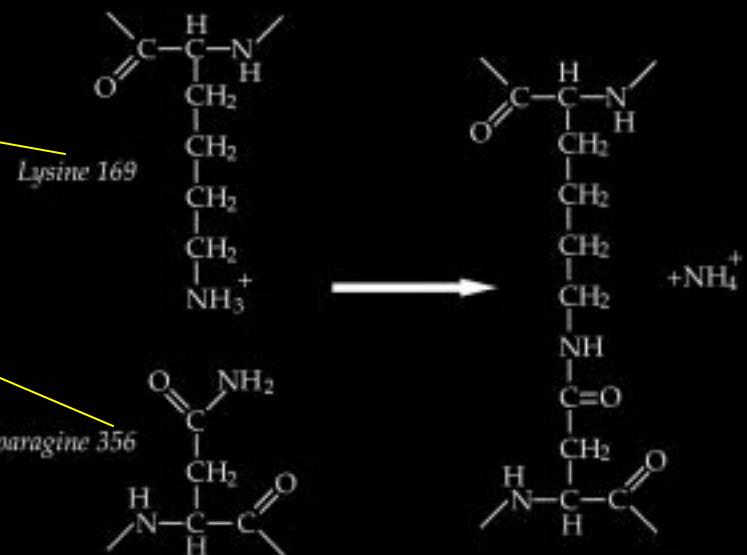
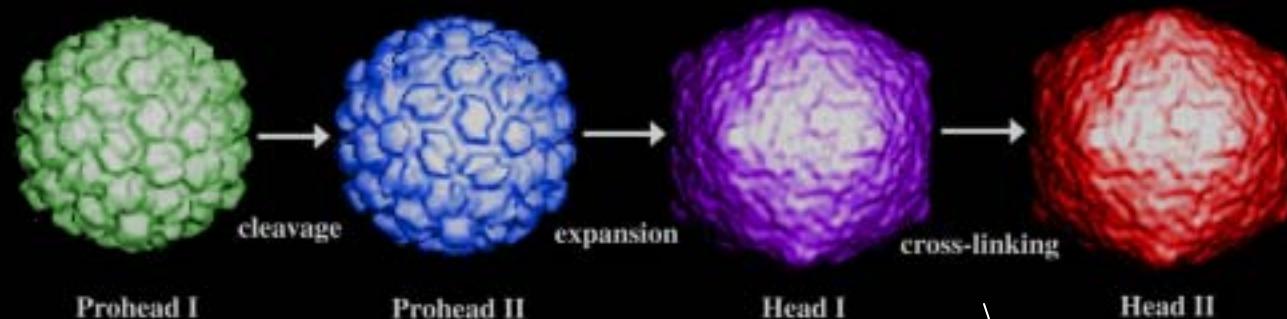
420 Sequence Identical Subunits

Capsid Subunit





Isopeptide Cross-link

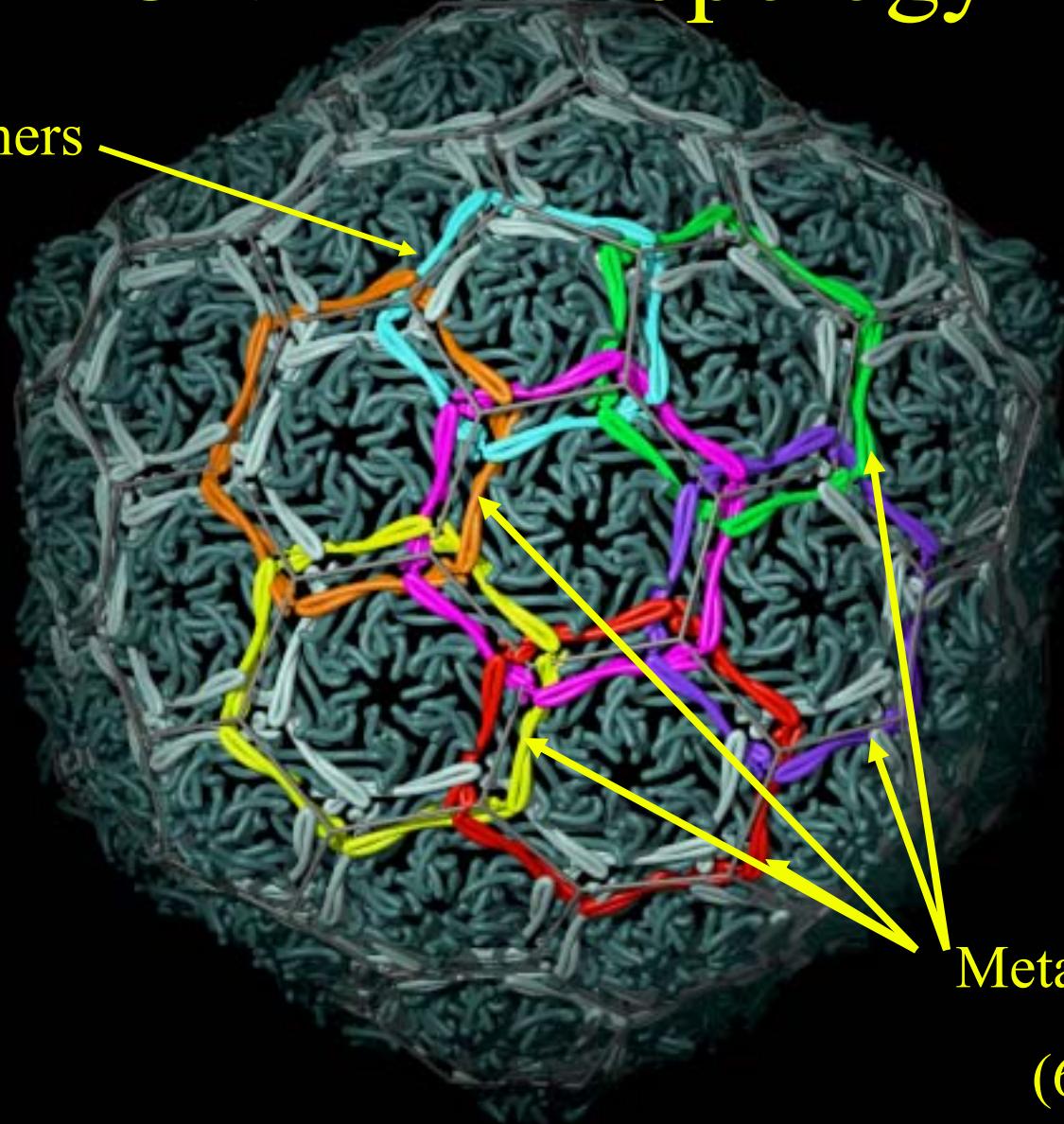


420 per Capsid

Chainmail topology

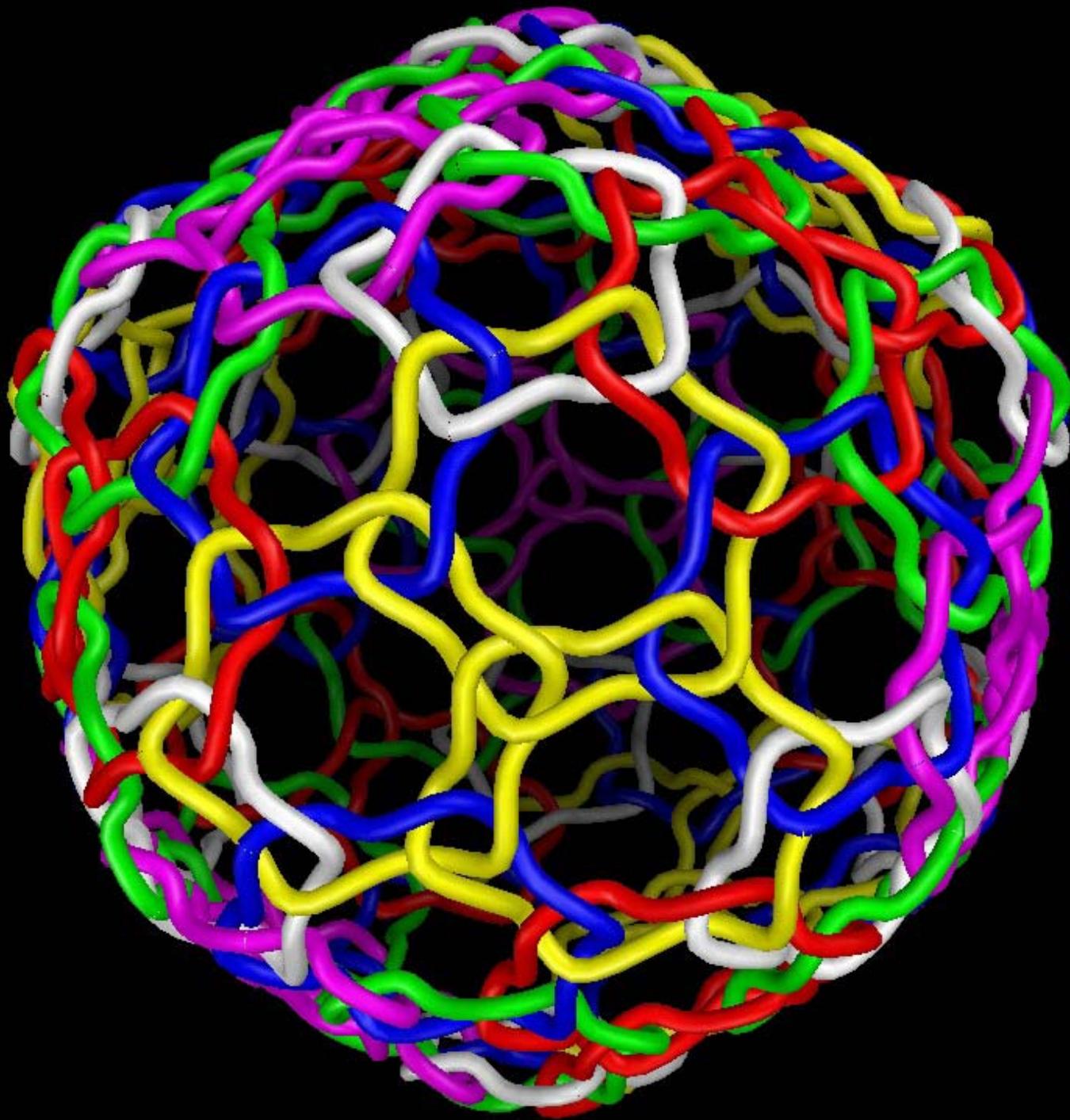
Meta-pentamers

(12 total)

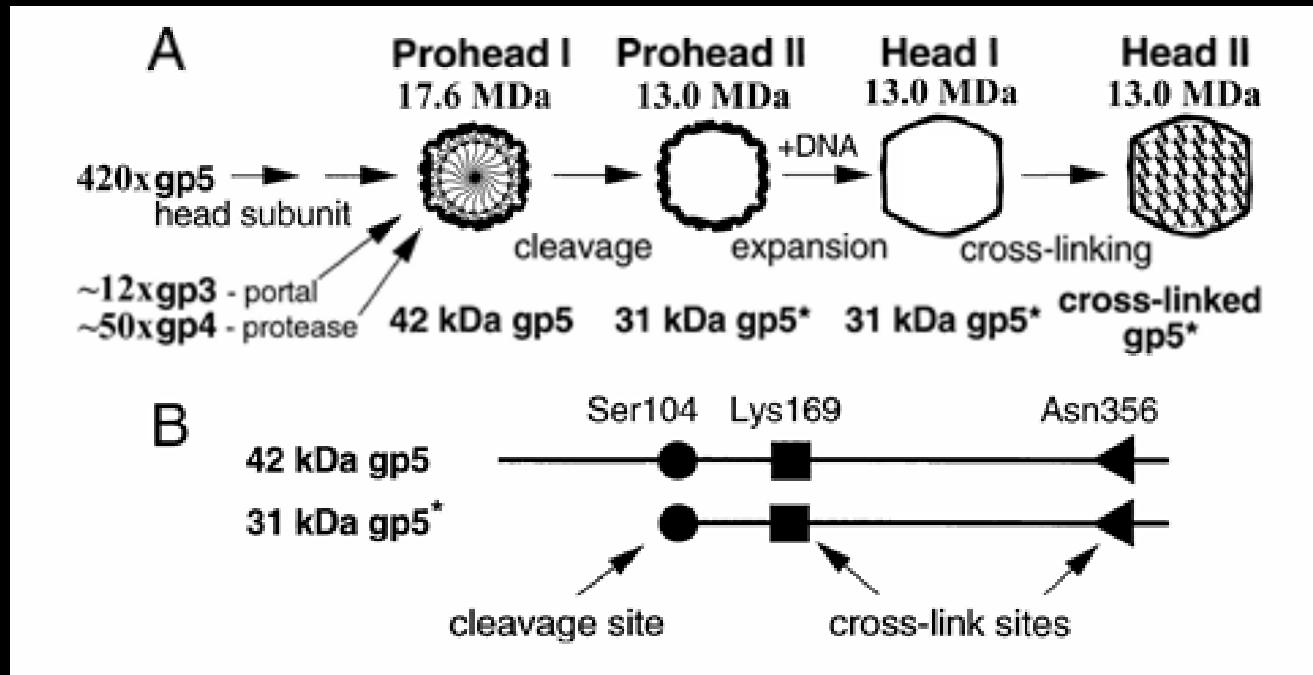


Meta-hexamers

(60 total)

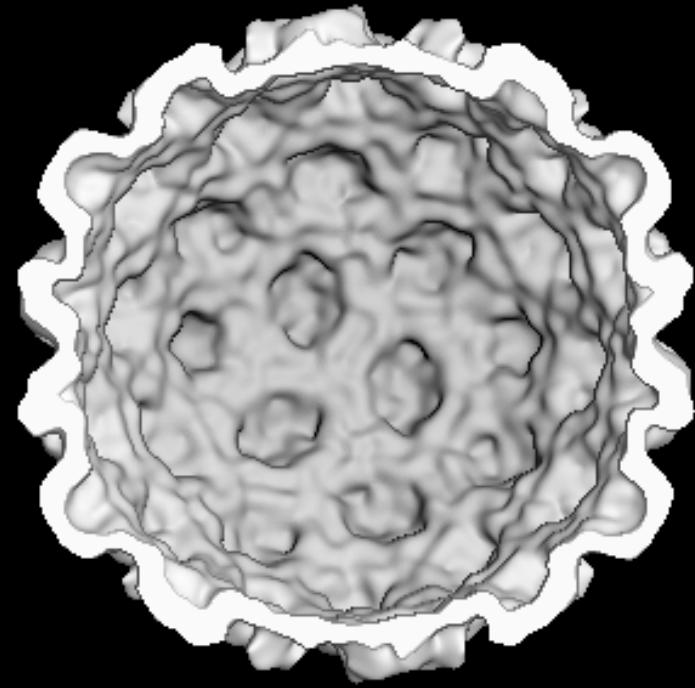
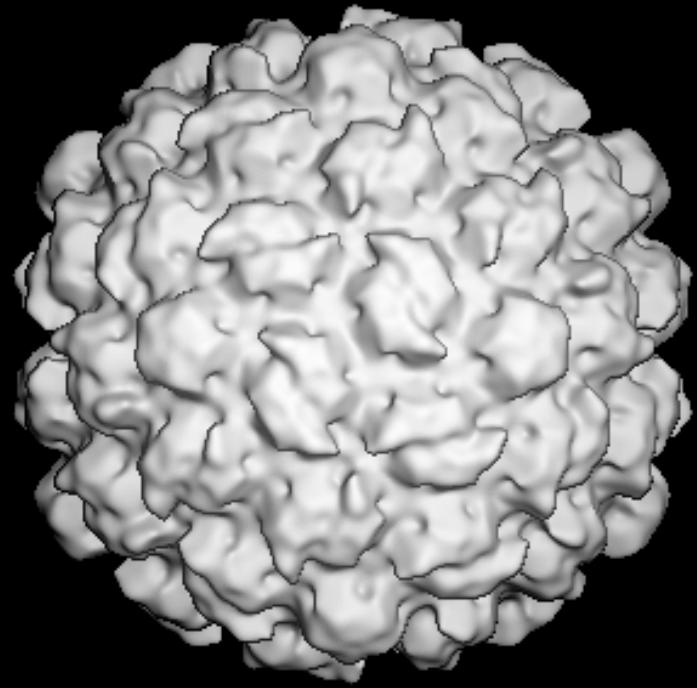


HK97 Morphogenesis



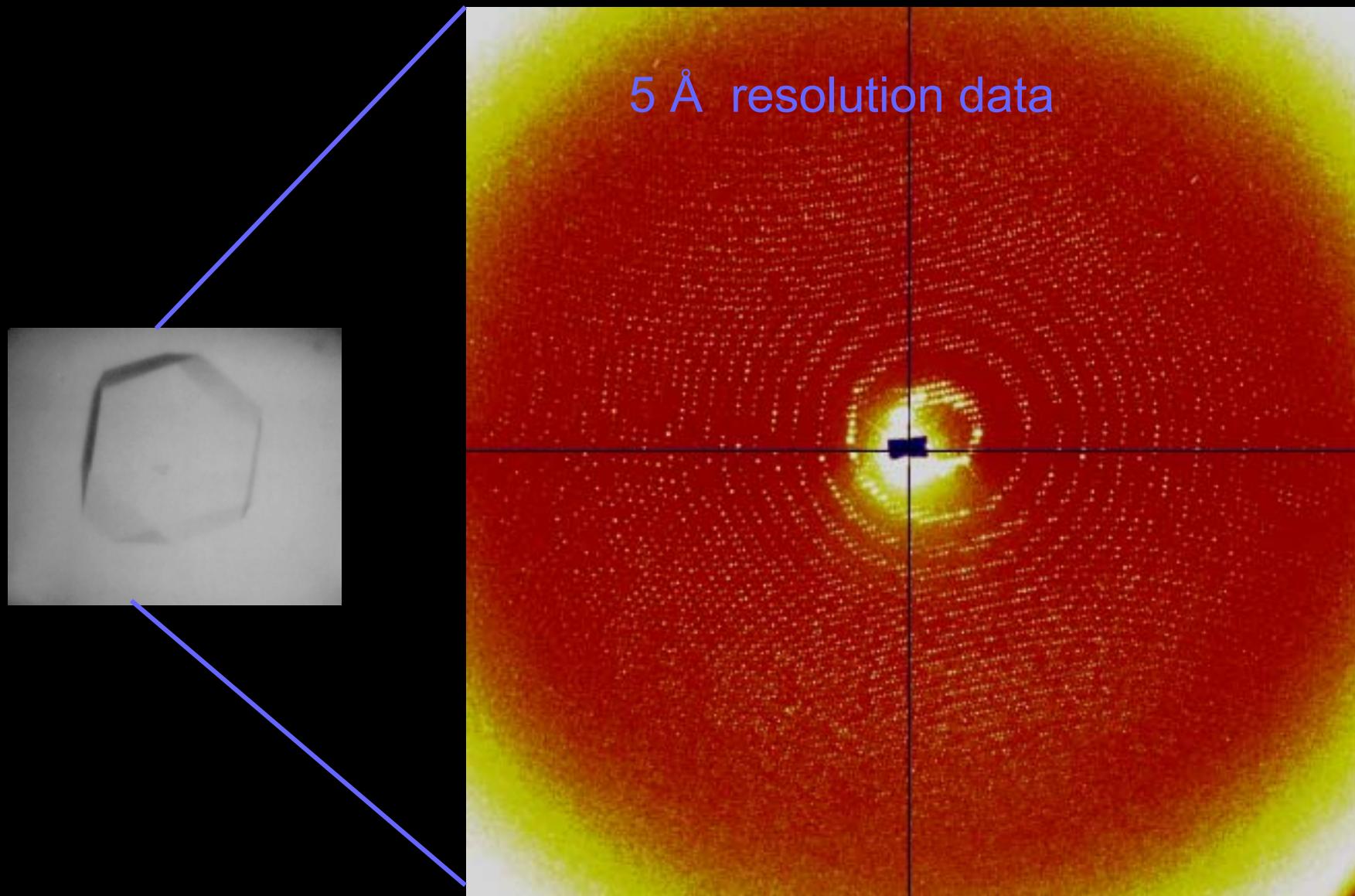
The morphogenesis of HK97 involves several metastable capsid states.:.

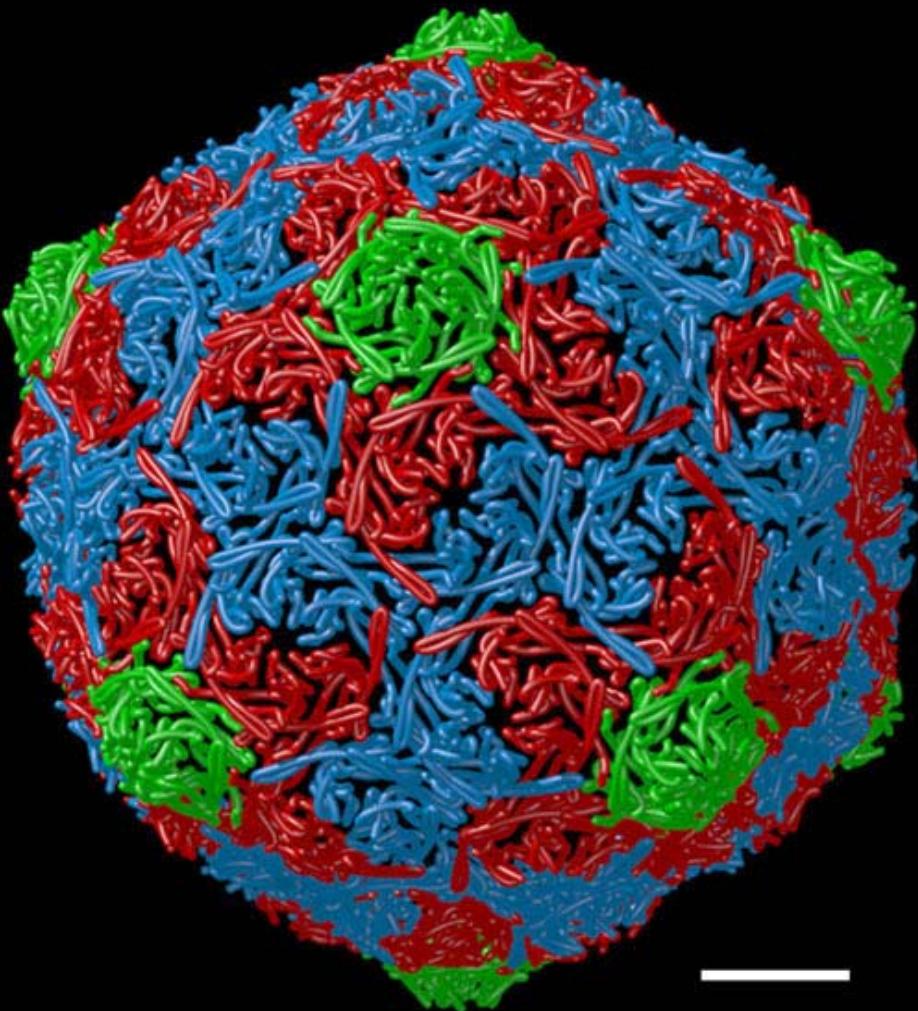
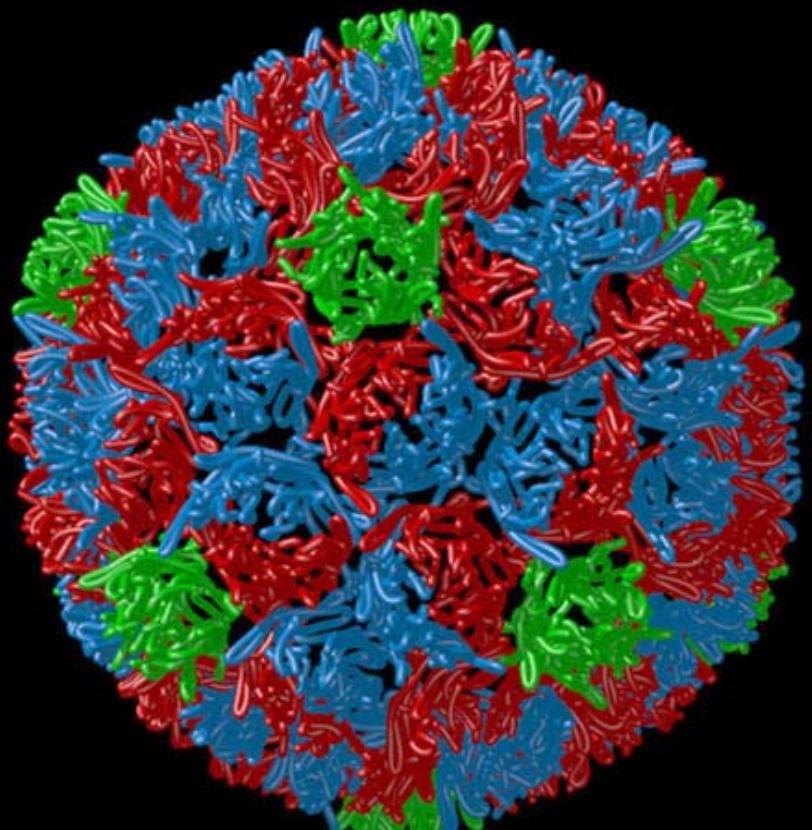
1. Capsid morphological unit = pentons (5 coat proteins) and hexons (6 coat proteins)
 2. 420 subunits arranged as 12 pentons + 60 hexons
 3. Prohead I contains 420 coat proteins, 12 portal proteins, 50 proteases
 4. Prohead II: 420 subunits of coat protein minus the N-terminal 103 residues
 5. Head II is the very stable, final state: molecular chainmail.

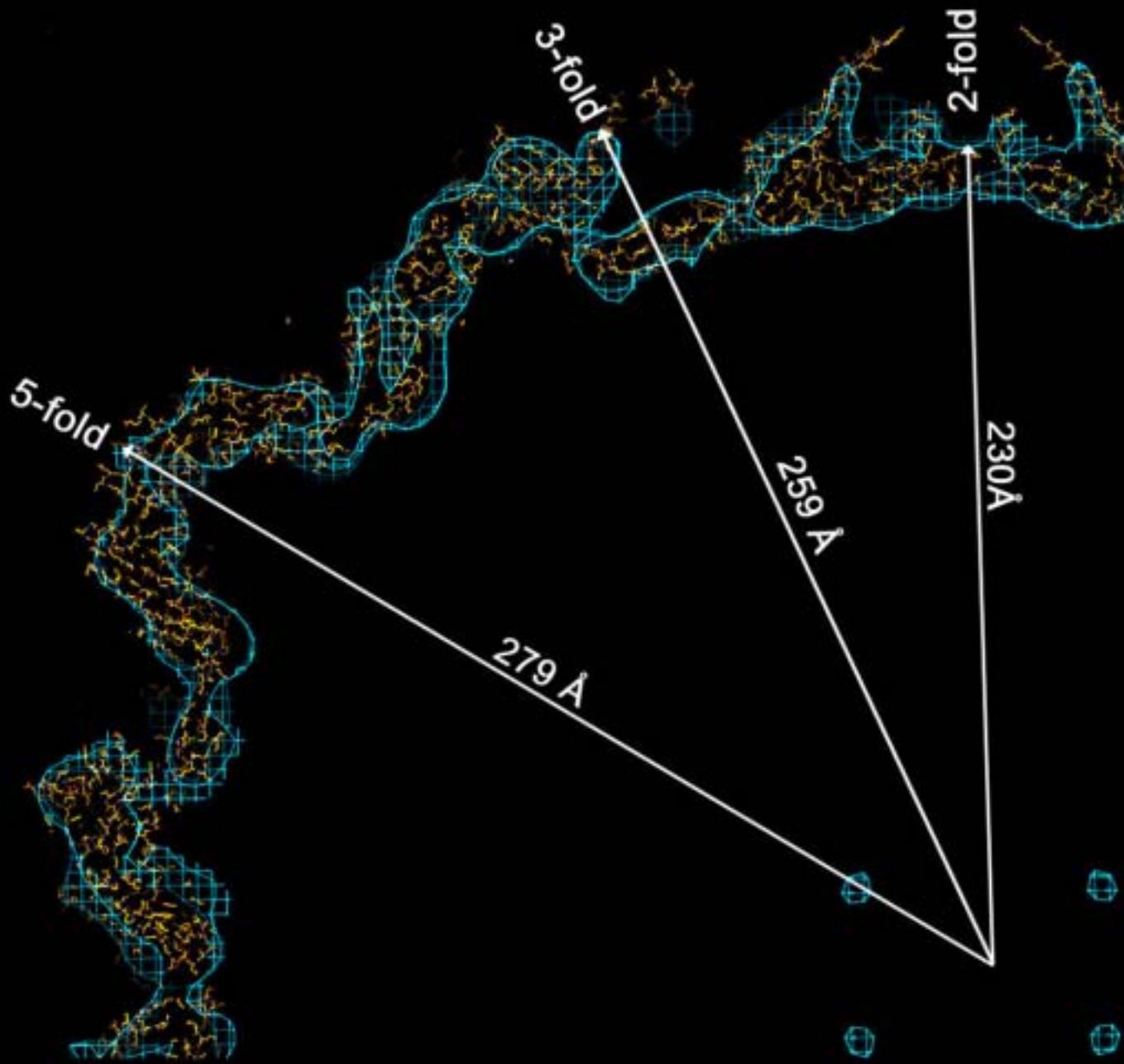


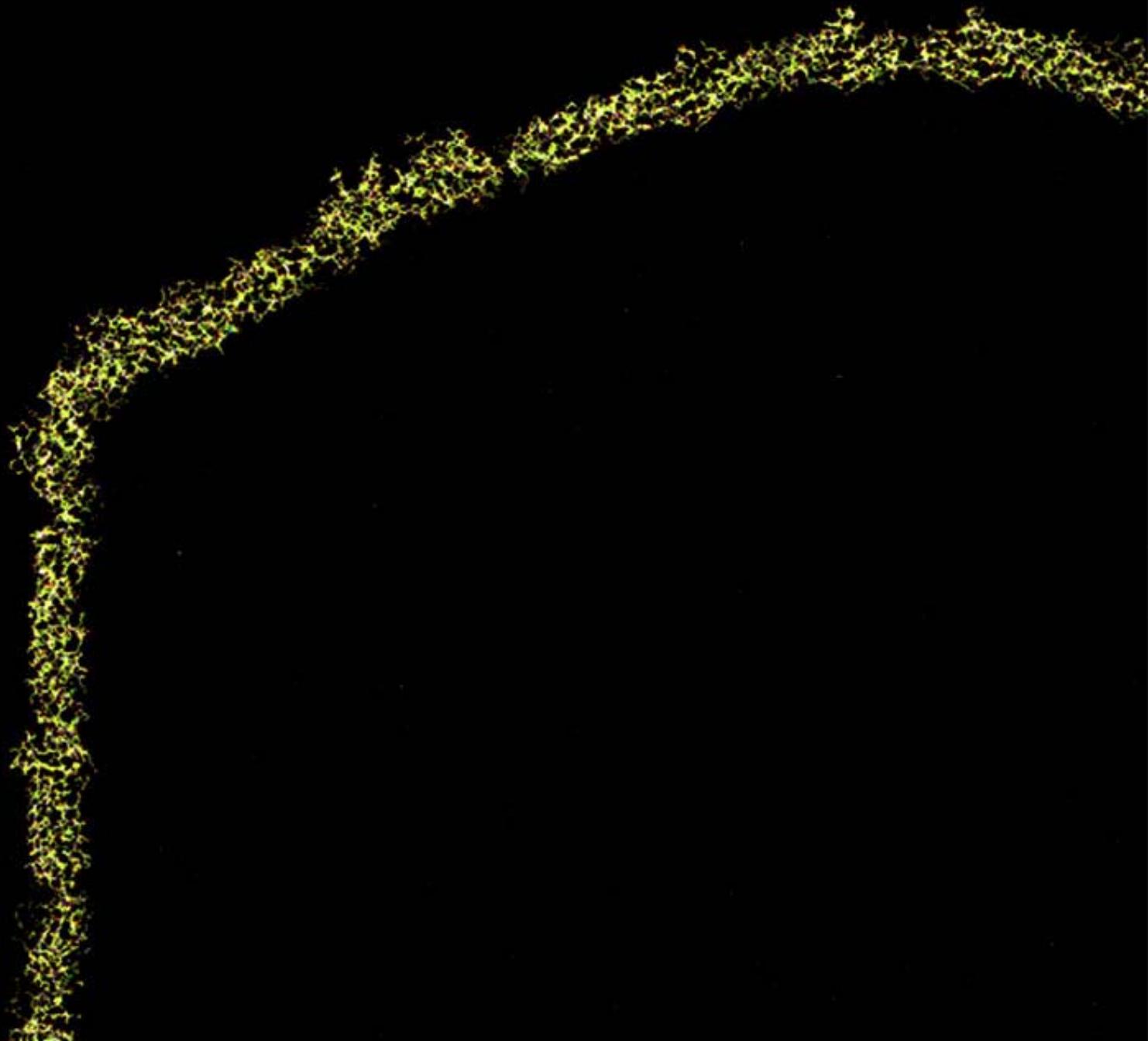
HK97 Procapsid II 20 \AA resolution cryoEM reconstruction
Conway et al. 1995

Prohead II crystals

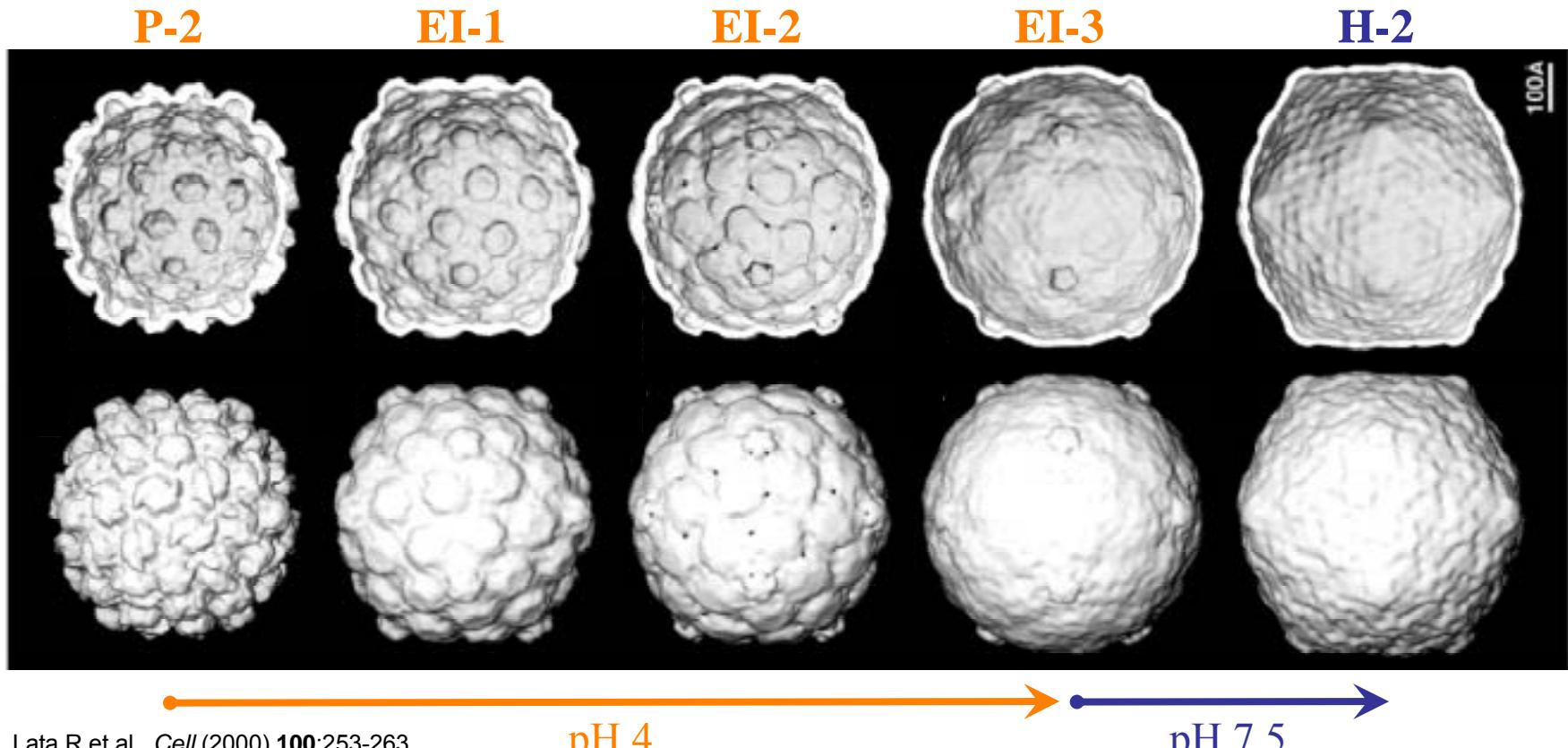








Acid-induced Maturation of HK97 Phage



Lata R et al., Cell (2000) 100:253-263.

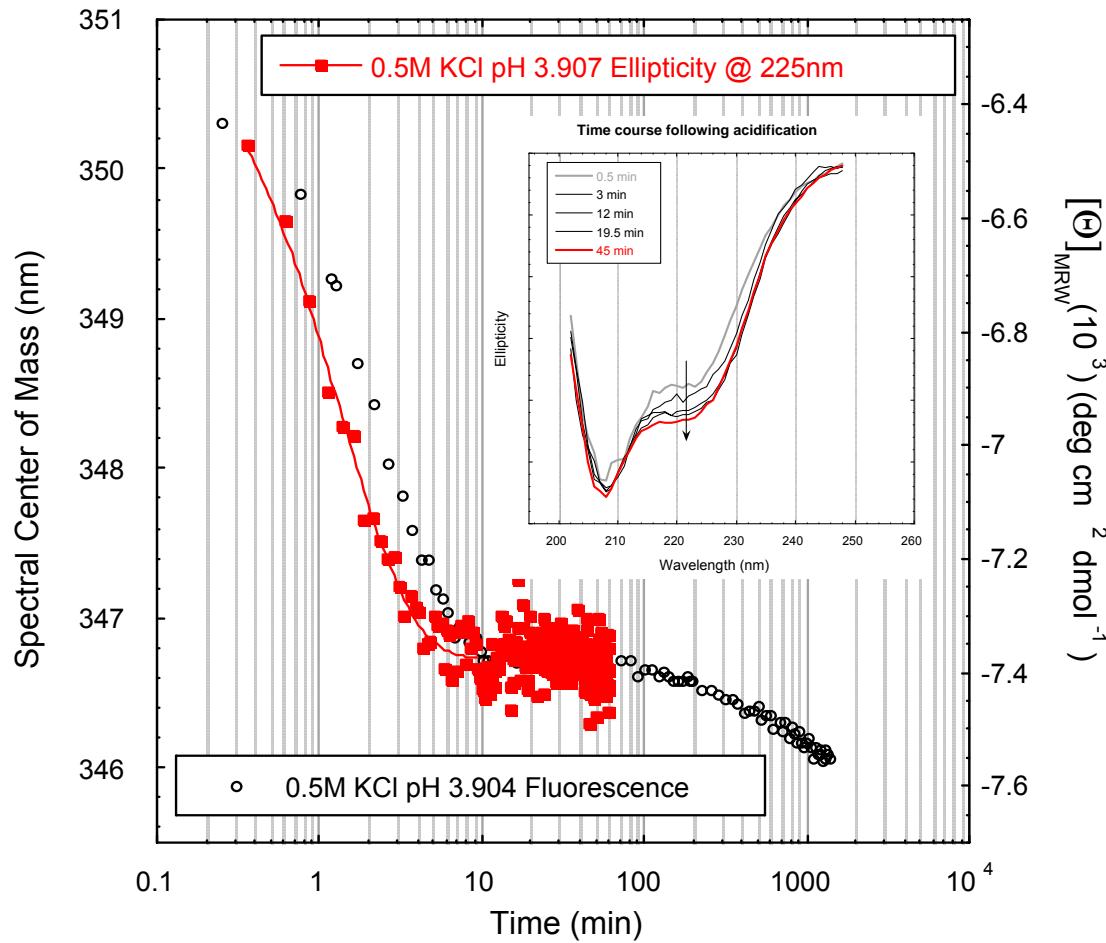
pH 4

pH 7.5

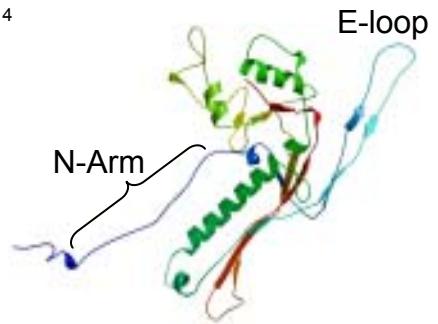
- *In vivo* maturation is driven by DNA-packaging.
- *In vitro* P-2 is metastable and maturation can be triggered *via* many factors including exposure to acidic pH.
- Multiple metastable expansion intermediates appear sequentially.

QuickTime™ and a Animation decompressor are needed to see this picture.

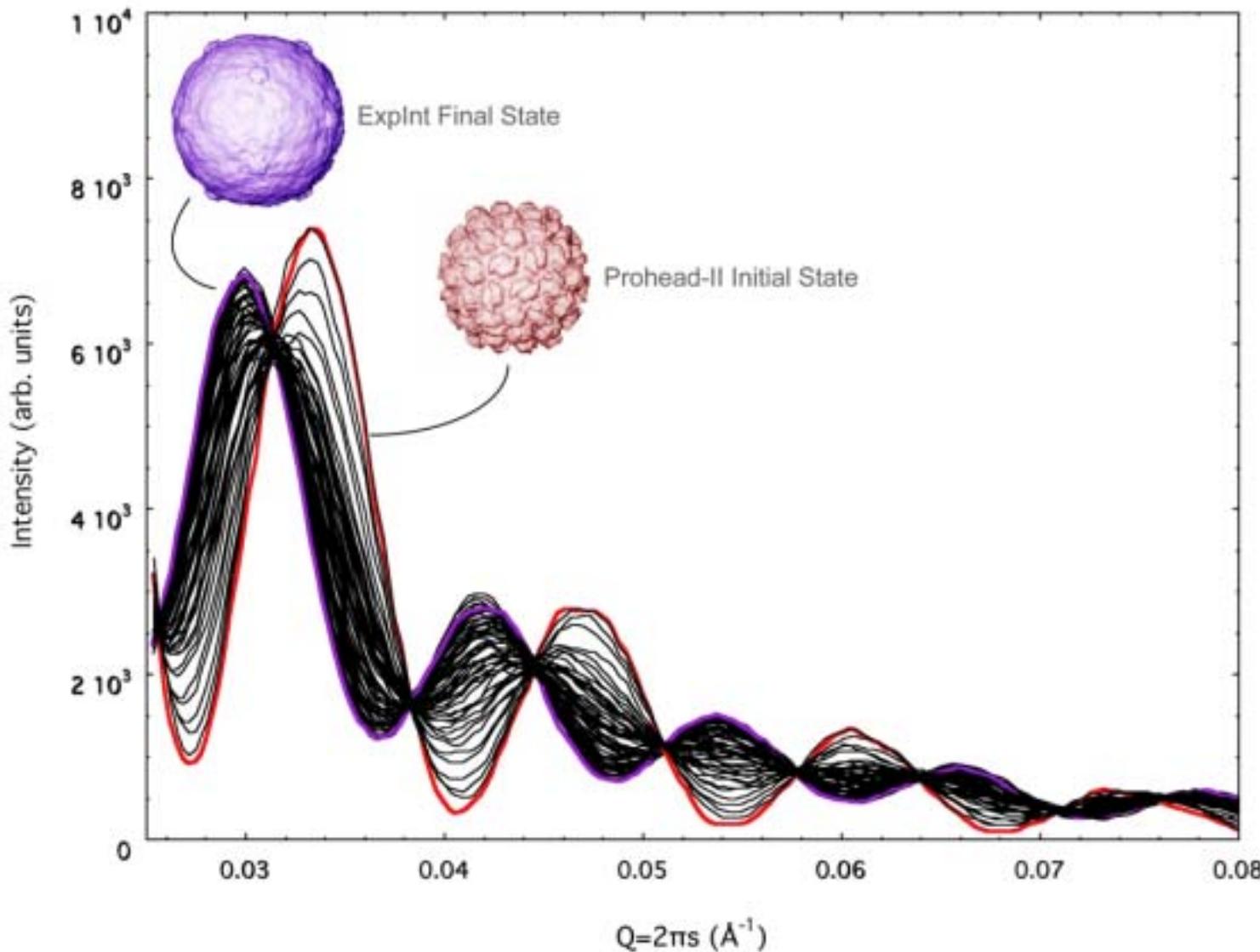
Changes in secondary structure probed by CD PRECEDE initial fluorescence phase



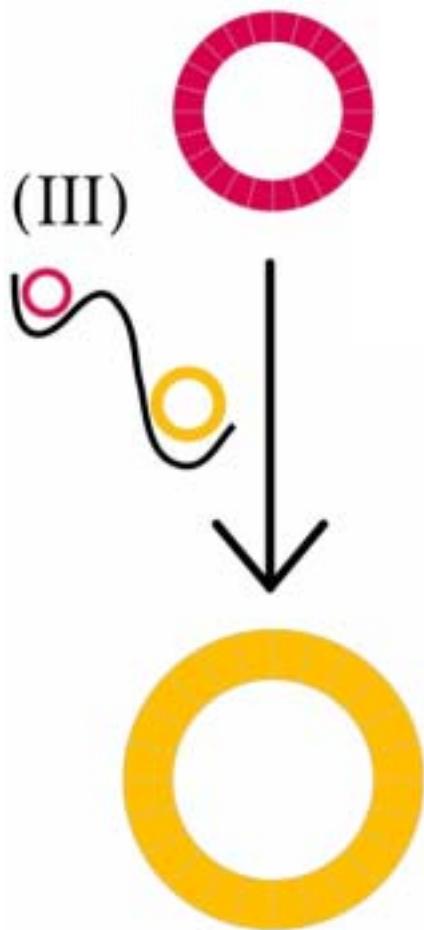
- Conversion of N-Arm from random coil to β -strand?

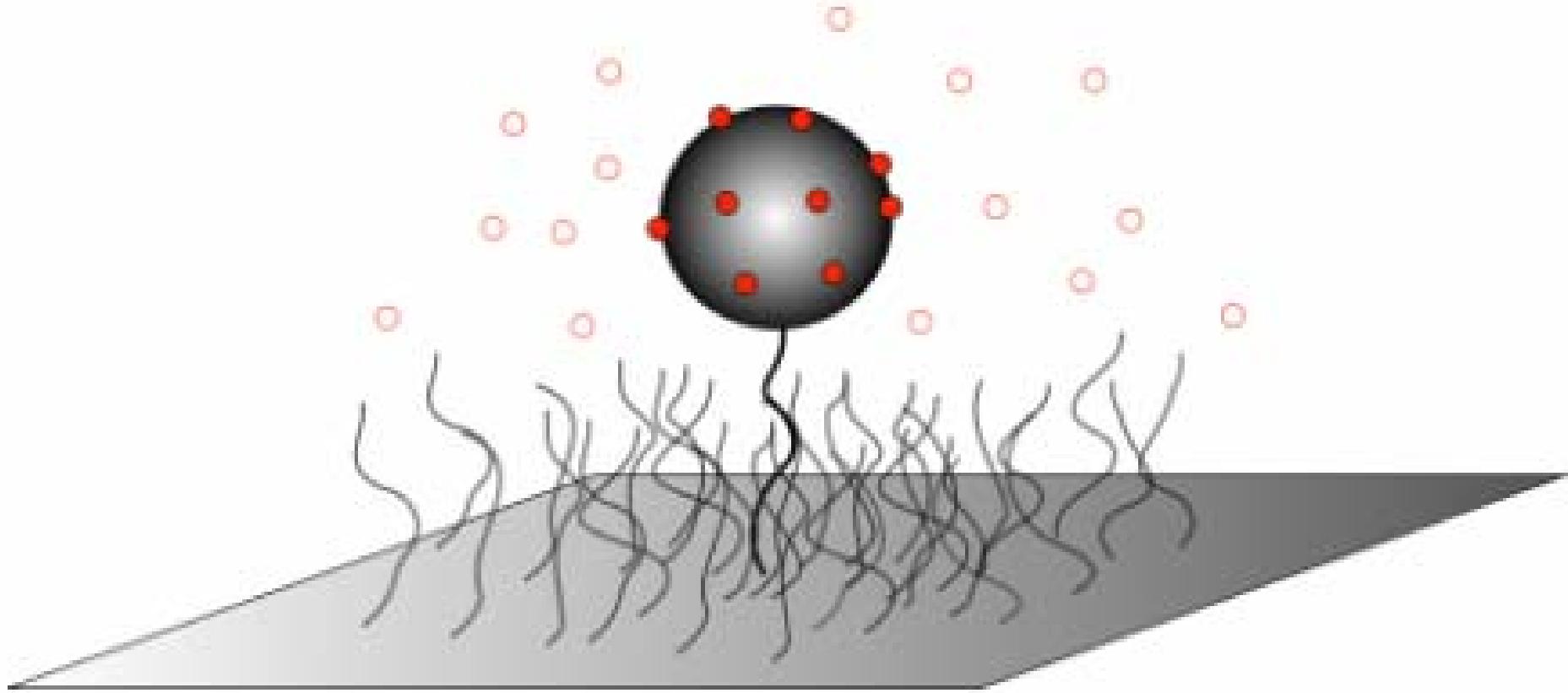


Isosbestic (isoscattering) points suggest expansion is two-state

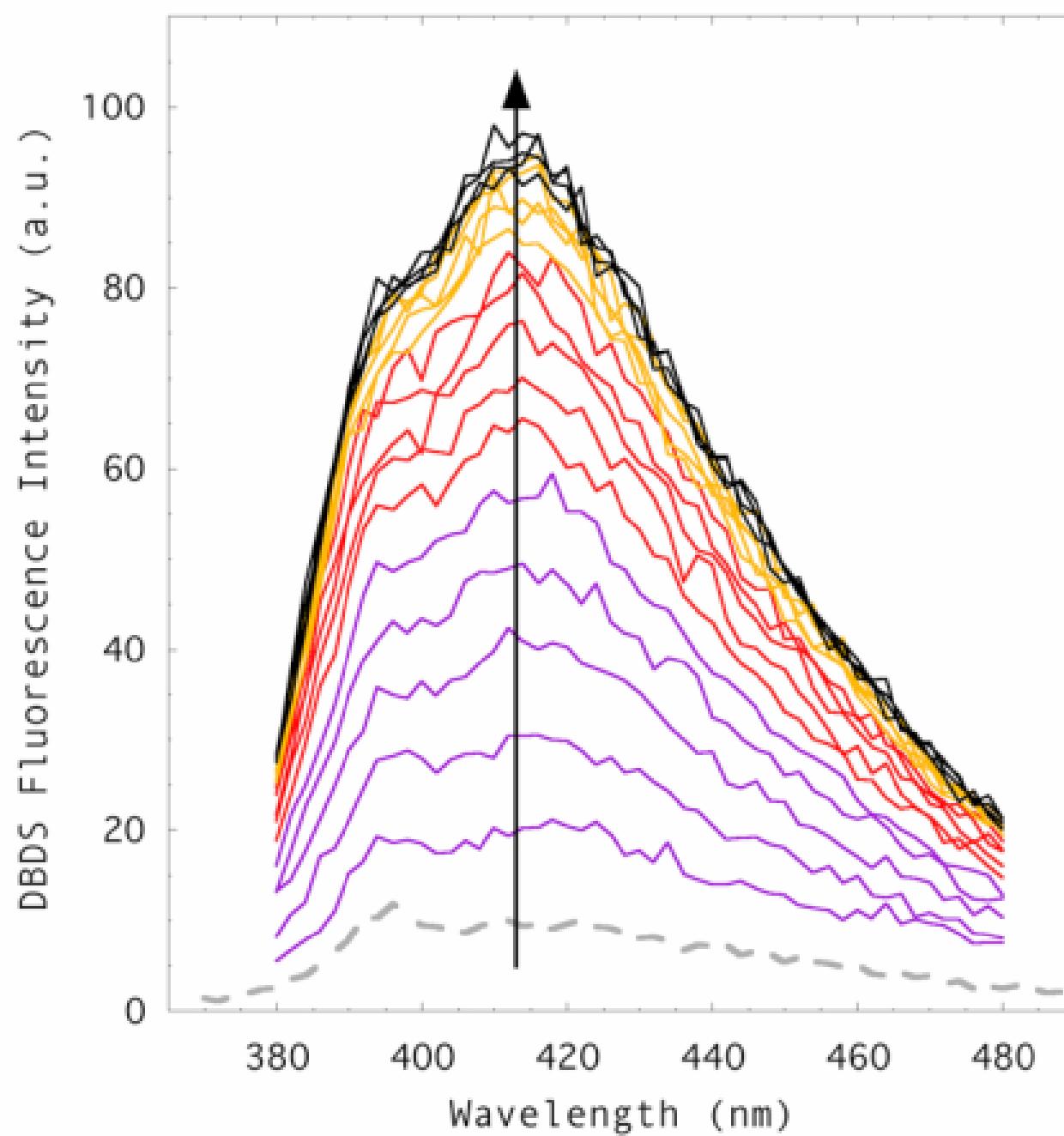


Whole particles hop over a single energy barrier

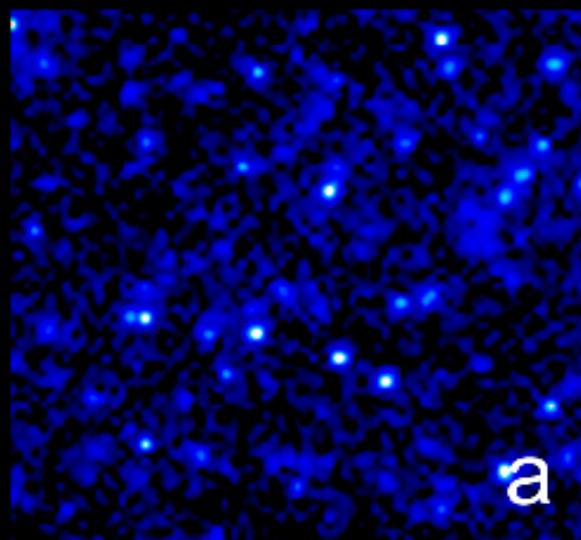




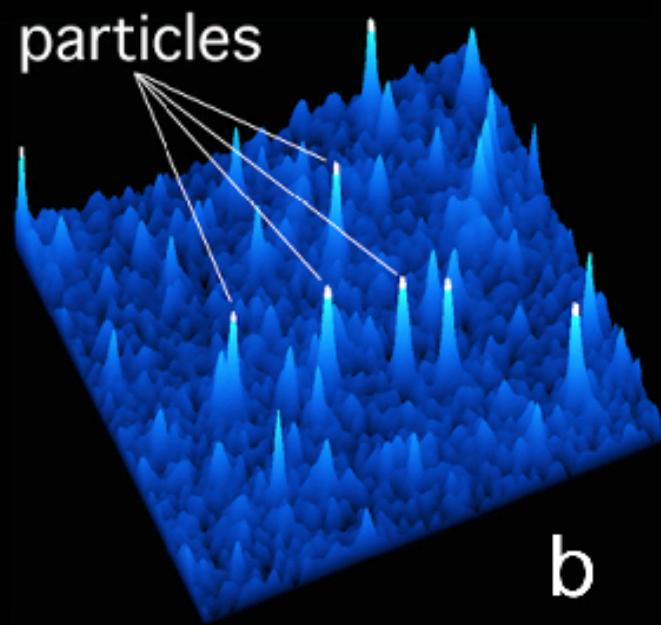
Time-resolved DBDS Fluorescence from acidified HK97 Prohead-2
Ex=348nm; Curves are spaced 1 minute apart



Single Particle Virus Dynamics

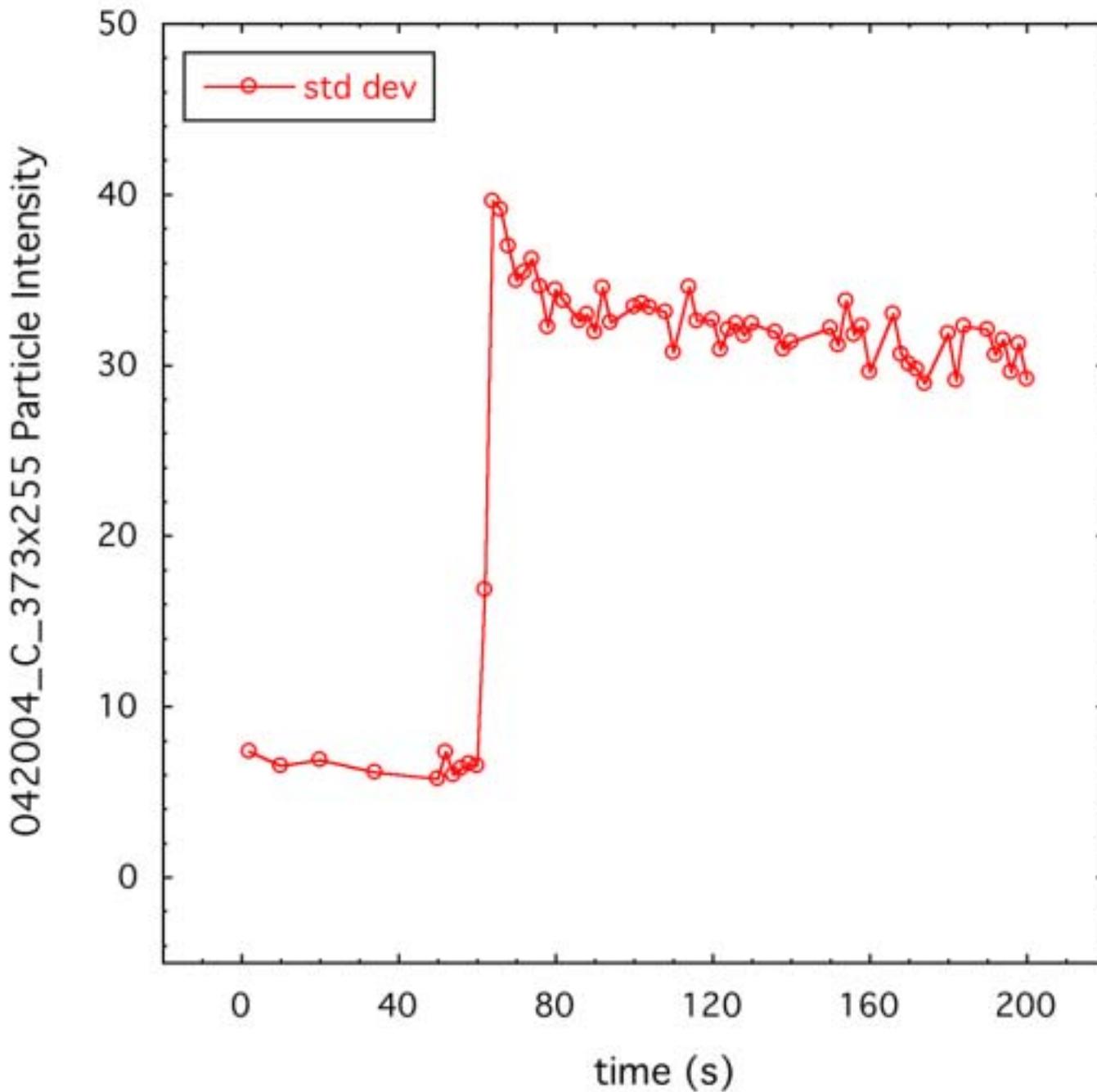


a

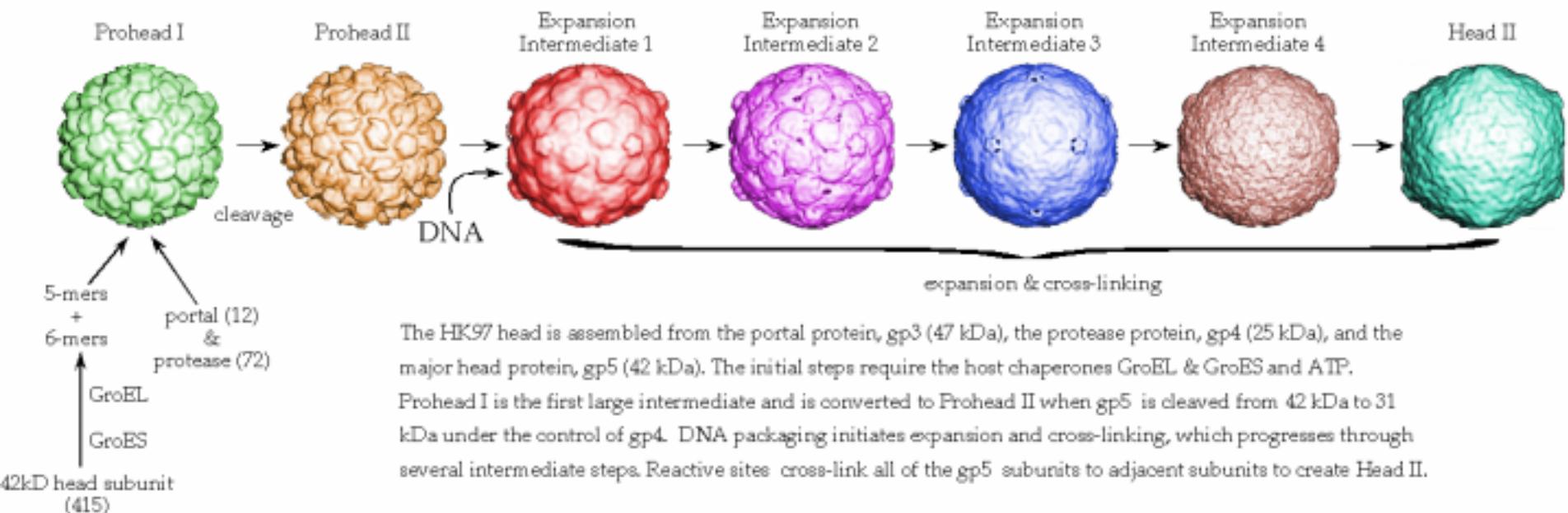


b

timetestC intens data



HK97 Head Assembly & Maturation Pathway



The HK97 head is assembled from the portal protein, gp3 (47 kDa), the protease protein, gp4 (25 kDa), and the major head protein, gp5 (42 kDa). The initial steps require the host chaperones GroEL & GroES and ATP. Prohead I is the first large intermediate and is converted to Prohead II when gp5 is cleaved from 42 kDa to 31 kDa under the control of gp4. DNA packaging initiates expansion and cross-linking, which progresses through several intermediate steps. Reactive sites cross-link all of the gp5 subunits to adjacent subunits to create Head II.

HK97 References

- Lata R, Conway JF, Cheng N, Duda RL, Hendrix RW, Wikoff WR, Johnson JE, Tsuruta H, Steven AC. 2000. Maturation dynamics of a viral capsid: visualization of transitional intermediate states. *Cell* 100:253-63
- Wikoff W, Liljas L, Duda R, Tsuruta H, Hendrix R, Johnson J. 2000. Topologically linked protein rings in the bacteriophage HK97 capsid. *Science* 289:2129-2133
- Conway J, Wikoff W, Cheng N, Duda R, Hendrix R, Johnson J, Steven A. 2001. Virus maturation via large subunit rotations and local refolding. *Science* 292:744-748
- Helgstrand, C., Wikoff, W., Duda, R., Hendrix, R., Johnson, J., and Liljas, L. 2003. The refined structure of a protein catenane: the HK97 bacteriophage capsid at 3.44 Å resolution. *J. Mol. Biol.* 334:885-899
- Wikoff, W., Che, Z., Duda, R., Hendrix, R., and Johnson, J. 2003. Crystallization and preliminary crystal analysis of a dsDNA bacteriophage capsid intermediate: Prohead II of HK97. *Acta Cryst. D* 59:2060-2064.
- Gan, L., Conway, J., Firek, B., Cheng, N., Hendrix, R., Steven, A., Johnson, J., and Duda, R. 2004. Control of cross-linking by quaternary structure changes during bacteriophage HK97 maturation. *Molecular Cell* 14:559-569.
- Lee, K. K., Gan, L., Tsuruta, H., Hendrix, R. W., Duda, R. L., and Johnson, J. E. 2004. Evidence that a Local Refolding Event Triggers Maturation of HK97 Bacteriophage Capsid. *J Mol Biol* 340:419-33.